INTRODUCTION

Pesticides are mainly responsible for water pollution hence non target organisms in contaminated water bodies come in continuous and direct contact with pesticides. Pesticide after penetrating in the body, affect ultrastructure of cell organelles like mitochondria, endoplasmic reticulum etc. They restrict the energy generating enzymatic activities of TCA cycle. It may result in imbalance of entire physiological equilibrium and various kinds of complications. Pollutants alter cellular functions, ultimately affecting physiological and biochemical mechanisms of animals (Satyaparmeswara et. al., 2006). Cavin, (1994) has reported that pesticide cause biochemical alterations such as inhibition of enzymes, metabolic disorder, genetic damage, hypertension and cancer due to physiological differences.

The mode of action of pesticides is subjected to their chemical properties. The sum of physiological changes generated by particular pesticide seems to be the characteristics of those pesticides. Many investigators reported a variety of wreckage in various metabolic processes in different species exposed to different kinds of pollutants (Langstone, 1986). Alteration in the life processes of organisms are the defensive phenomenon to combat with the stressful situation. In spite of detoxification mechanism in the organism to get rid of from foreign substances, toxicants induce adverse effects. To predict the frequency of toxic effects of various toxicants, it is necessary to understand these mechanisms properly.

It is well-known that every living organism has its own detoxification mechanism to get rid of foreign substances in its body, however if toxic substance encountered in higher concentration they are bound to bring severe adverse effect on the organism. Such
an effect may be at cellular or even at molecular level but ultimately it would lead to physiological, pathological and biochemical disorders that may prove total to the organism (Patil et. al. 1989; Jain 2000.)

Recent understanding of different biochemical processes has proved useful in determining the mechanism of toxicity of different toxicant as also in unfolding the adaptive protective mechanism of the body to fight the toxic effect of the pollutant (Thateyush et. al., 1987). Besides it is also now felt that some of the biochemical alternation occurring in the body gives the first indication of the stress in the organism and hence effect on the part of the pollution (Ruparelia et. al., 1992).

Qualitative and quantitative study of changes in major biochemical components of organisms such as proteins, amino acids, ascorbic acids, fats, vital enzymes, DNA and RNA 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 vigor of an organism.

Investigation regarding the physiological and biochemical changes in non target aquatic species such as molluscs is insufficient. Fresh water bivalves amongst the molluscs are economically important hence, an attempt is made to investigate the effect of indoxacarb and thiamethoxam on the biochemical composition of different tissues of fresh water bivalve, Parreysia cyllindrica. Some basic mechanisms of the mode of action of pesticides were studied on these model animals which can be applicable to other higher forms. Vital organs viz. mantle, foot, gills, digestive glands and whole body tissues are used to determine the changes in protein, amino acid, ascorbic acid, DNA and
RNA content of their tissues after acute and chronic treatment to indoxacarb and thiamethoxam in the fresh water bivalves *Parreysia cylindrica*.

**Proteins:**

Proteins are one of the proximate constituents of the body. The term protein was first coined by Mulder in 1940. Proteins are essential constituents of protoplasm. Protein acts as growth material for organism. It is also a source of fuel in the body. As a constituent of cell membrane, proteins regulate the process of interaction between intra and extra cellular media.

Any undesirable change in the environment affects the protein level by changing the physiology of organism. Pesticides are known to disturb the protein metabolism in the body of organism. Nagy *et. al.*, (1981) reported that the pesticides interfere with protein synthesis and degradation, resulting in alteration of dynamic equilibrium. The pesticides affect the activity of biologically active molecules such as amino acids, co-enzymes and other proteins containing sulphur, phosphorus and affect physiological processes in tissues (Ghosh and Chatterjee 1985). Biochemical changes induced by pesticide stress lead to disturb the metabolism, inhibition of important enzymes, retardation of growth and reduction of fecundity and longevity of organism (Murty, 1986).

Reports are available regarding the impact of pesticides on protein metabolism in different organism. The biochemical variation in marine bivalve *Mytilus edulis* was studied by William, (1969). Young (1970) suggested that, dynamic equilibrium mechanism in the internal environment of organism changes the protein content of cell periodically by the degradation and synthesis. Malathion shows decrease in total protein content of the pelecypod, *Lamellidens marginalis* (Kabeer *et. al.*, 1971). Shakoori *et. al.*,
*cylindrica* after cypermethrin exposure. Parate and Kulkarni (2003) reported changes involved in total protein profile in the muscles and gills of the crab, *Paratelphusa jacquimontii*, exposed to cypermethrin at different experimental conditions.


Mohanty *et. al.*, (2005) analyzed and compared protein profiles in different tissues namely, gills, foot and mantle of two fresh water bivalves, *Lamellidens corrianus* and *Lamellidens marginalis* and found protein markers which helps to study the molluscan taxonomy. Kharat *et. al.*, (2009) observed decrease in total protein content was possibly due to stress condition caused by toxicity of tributyltin chloride on protein metabolism or due to enhanced proteolytic activity as a consequence of increased metabolic demands following exposure to the toxic stress of tributyltin chloride. Thenmozhi *et. al.*, (2009) observed in crab stress induced alteration in the carbohydrate protein and lipid content decreased when exposed to higher concentrations because of their utilization to meet the energy requirement during the stress caused by “Cattle shed effluent”. Kamble *et. al.*, 

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(2010) studied the biochemical changes in the protein, glycogen, lactic acid and cholesterol, in the tissues like gills, hepatopancreas, gonads, muscle, mantle and foot fresh water bivalve.

**Free amino acid:**

Amino acid is important metabolic compound; they serve as the major energy source. Amino acid can be divided in two major groups, those that can use in protein synthesis and those that have other functions. They conform to particular general structure-NH-CHRCOOH where R is any one a number of organic side chain having some or all of carbon, hydrogen, oxygen, nitrogen and sulphur atoms. Some of the side chains are quite complex, forming ring structures, for example tryptophan.

The 20 standard amino acids found in proteins can be divided further in two groups, the essential and non-essential amino acids. Essential amino acids are defined as those amino acids that cannot be synthesized by an animal. As a result, they must be obtained from their diet. The non-essential amino acids are those that the animal can synthesize from other compounds. Amino acids suffer one of the three fates in an animal. They can be used for protein synthesis, they can be subjected to structural change to produce another compound or they can be degraded for energy.

The breakdown of amino acids can incorporate three stages which are: 1) deamination (removal of the amino group) which is either converted to ammonia or transferred to become the amino group of a glutamic acid molecule; 2) Conversion of amino acid carbon skeletons (the α- keto acids produced by deamination) to citric acid cycle intermediates; and 3) incorporation of ammonia into urea.
In all animals ammonia build-up in the blood is toxic. In order to prevent this happening in higher vertebrates ammonia is converted to urea for excretion in the urine.

Amino acids are quite soluble and occur free in solution in the intra and extra cellular body fluids. This pool of dissolved amino acids is termed as free amino as (FFA) as opposed to the polymerized amino acids. Dietary amino acids imbalances lead to increased amino acid oxidation and decreased food conversion efficiency.

Amino acids can be used for protein synthesis, they can be subjected to structural change to produce another compound or they can be degraded for energy. Amino acids are important metabolic compound which on consumption are degraded for major energy source. Apart from providing a major energy source, amino acid is used as components of proteins. Synthesis and degradation of protein occur continuously in tissues. When the rate of protein synthesis is greater than the protein degradation the animal is able to grow.


Very few report are available pertaining to the action of pesticides and the changes in amino acid levels in invertebrate in general and almost none in the fresh water bivalve, *Parreysia cylindrica*.

**L-Ascorbic acid:**

L-Ascorbic acid (Vitamin C) being water soluble is daily excreted. Ascorbic acid is a hexose derivative and closely resembles monosaccharides in structure. The acidic property of vitamin C is due to the enolic hydroxyl groups. There are two enantiomers of ascorbic acid. The naturally occurring vitamin ‘C’ is L-ascorbic acid. D-ascorbic acid has the antagonistic action to vit. C. L-ascorbic acid was first isolated by a Nobel Prize winner (1937) Szent – Gyorgyi a physician and biochemist in 1928. It was fist synthesized by Reichstein in 1933. L-ascorbic acid is an enediol isomer of 2 – keto – L – gulonolactone having a configuration similar to that of L – glucose. Its empirical formula is \( \text{C}_6\text{H}_8\text{O}_6 \). It is white crystal, water soluble and heat – labile. It can be oxidized easily at 100 °C in presence of oxygen. Glucose is the precursor of ascorbic acid. Ascorbic acid can be oxidized into dehydro – L – ascorbic acid. This reaction is reversible. Both forms of ascorbic acid are physiologically active.

It plays vital role as an antioxidant that serves protective function against oxidative damage in tissues. In animals during stressed condition ascorbic acid level is altered which indicates protective role of ascorbic acid in detoxification. Antioxidant
property of ascorbic acid helps to prevent free radical formation from water soluble molecules, which may causes cellular injuries and disease. It is strong reducing agents. Ascorbic acid is found in plants, animals and single-cell organisms. All living animals make it, eat it or die from scurvy due to lack of it. Reptiles and older orders of birds make ascorbic acid in their kidneys. Recent orders of birds and most mammals make ascorbic acid in their livers where the enzyme L-gulonolactone oxidase is required to convert glucose to ascorbic acid Stone Irwin (1972).

Beside this significance ascorbic acid takes part in variety of other biochemical functions such as, biosynthesis of amino acid carnitine and the catecholamines that regulate the nervous system. It helps in degradation of histamine which is the inflammatory component of many allergic reactions. Asthmatic patients treated with vitamin ‘C’ had experienced much less difficulty in breathing (Mirc and Haxhiu, 1991).

In chronic brucellosis vitamin ‘C’ was found to be effective in setting the striking immune responses against the infection (Borah 1989). Dieter and Breitenbach (1971) suggested that, lymphoid tissue regeneration and its differentiation takes place under the influence of ascorbic acid. Interferons get enhanced in circulatory system after the ingestion of ascorbic acid through diet (Siegel, 1974).

Animal study reveals that, vitamin ‘C’ reduces clump of platelets by stimulating synthesis of prostacyclin and interfere with platelets activities. It can also prevent or reverse the plaque formation caused due to high cholesterol diet. According to current evidence ascorbic acid may be mainly beneficial in reducing the risk of developing cancer rather than in therapy (Gaby and Singh, 1991). It is also found that, ascorbic acid
has an important role in protecting the lens. In combination with vitamin E, vitamin ‘C’ may provide protection against cataracts (Robertson et. al., 1991).

Ascorbic acid acts as detoxifier and may reduce the effect of toxicant through its anti-oxidant property. Its role in detoxification and immune system may protect the body from various toxic pollutants in environment. Possibly, ascorbic acid has some role in enhancing the resistance of animals against different toxins and stress conditions. But the study regarding the change in ascorbic acid content in molluscs exposed to various toxins and stress situation is inadequate and can be useful as an indicator for the study.

Ascorbic acid takes part in the synthesis of collagen and bone formation and in wound healing (Gould, 1963). Chinoy and Seethalakshmi (1977) reported that ascorbic acid has significant role in steriodogenesis in molluscs. It helps in tissue synthesis and growth processes and obviously coordinates rapid tissue repair in trauma or diseased condition (Halver, 1972). Ascorbic acid is necessary in the formation and maintenance of collagen, the basis of connective tissue, which is formed in skin, ligaments, cartilage, vertebral discs, joint linings, capillary walls, bones and teeth. It acts as radio protective agent in several tissues including reproductive organs by preventing radiation induced oxidation (Chinoy and Garg, 1978). It plays an important role in the process of hydroxylation, oxygenation and oxidation of corticosteroids (Chatterjee, 1967). It takes part in synthesis of collagen and maturation of red blood corpuscles (Talwar, 1980).

Pesticide causes stress to the aquatic organisms (Newell 1973), changes its metabolic activity and causes metabolic derangement in the living system. Pesticides due to its potential toxicity produce biochemical changes in the organs of animals (Sastry and Sharma, 1979).
The study regarding the change in ascorbic acid content in molluscs exposed to various toxins and stress situation is inadequate and can be useful as an indicator for the study.

Siddique (1967) observed increase in ascorbic acid level in liver, gonads and serum of Ophicephalus punctatus. Chitra and Ramanna Rao (1977) suggested variety of changes in blood glycogen and ascorbic acid levels at low temperature. Sinha et. al., (1978) proved rapid mobilization of fat by ascorbic acid and formation of glucose from fat. Somasundaram et. al., (1978) found increased level of ascorbic acid in various tissues of guinea pigs at tolerable concentration (0.0001 ppm. to 0.1 ppm.) of dieldrine. Ali and Ilyas (1981) reported alleviative role in dimecron exposed Channa gachua. Bhusari (1987) reported alteration in the ascorbic acid content in the tissues of fresh water fish, Barbus ticto on exposure of endosulfan and ekalux. Kulkarni et. al., (1988) estimated the effect of temperature and pH on ascorbic acid content of Indonaia caeruleus. Vedpathak (1989) reported rise in ascorbic acid levels with increase in temperature in Indonaia caeruleus. Zambare (1991) studied the effect of pollutants on ascorbic acid content in various tissues of fresh water bivalve, Parreysia cylindrica. Muley and Mane (1995) found increase in ascorbic acid content in hepatopancreas, mantle and gonads of fresh water bivalve, Lamellidens marginalis when exposed to various pH. Jadhav et. al., (1996) reported that acute and chronic treatment of carbaryl to Corbicula stiatella causes decrease in ascorbic acid content in mantle, foot, gills, digestive gland and whole body.

Waykar et. al., (2001) studied effect of cypermethrin on the ascorbic acid content in mantle, foot, gill, digestive gland and whole body tissues of fresh water bivalve Parreysia cylindrica. Gradual decrease in the levels of liver protein and liver ascorbic
acid due to proteolysis and liver glucose breakdown respectively was observed Desai and Sekhar (2002). Waykar and Lomte (2004) studied carbaryl induced changes in the ascorbic acid content in different tissues of fresh water bivalve *Parreysia cylindrica*. Pardeshi and Zambare (2005) studied ascorbic acid content in various tissues viz. mantle, foot, gill, gonad and digestive glands of the fresh water bivalve, *Parreysia cylindica* in connection with reproduction.

Perusal of literature shows that the ascorbic acid content in the tissue of fresh water bivalves has received little attention. Considering the multiple role of ascorbic acid and paucity of information on its level in the tissues of fresh water bivalve, and considering the impact of pesticides on ascorbic acid content, it is planned to study the changes in the ascorbic acid content during acute and chronic exposure to pesticides indoxacarb and thiamethoxam from the mantle, foot, gill, digestive gland and whole body of bivalve *Parreysia cylindrica*.

**DNA:**

DNA is the chemical basis of heredity and may be regarded as reserve bank of genetic information. In 1953 the molecular biologists J. D. Watson, an American, and F. H. Crick, an Englishman, proposed that the two DNA strands were coiled in a double helix. In this model each nucleotide subunit along one strand is bound to a nucleotide subunit on the other strand by hydrogen bonds between the base portions of the nucleotides. DNA is exclusively responsible for maintaining the identity of different species. Further, every aspect of cellular function is under control of the DNA as the genetic material carries information to specify mono acid sequences in proteins. Structurally DNA is linear polymer composed of monomer called nucleotides, i.e. four
nitrogen bases, two purines; adenine, guanine and two pyrimidine cytosine, thymine. Double helical structure of DNA consist of two polynucleotide strands that winds together to form double helical structure.

Nucleic acid contents are considered as an index of capacity of an organism for protein synthesis. Different hormones and stress conditions may exert control over synthesis, activity and break down of nucleic acids. The nucleic acid contents can cause alterations in genetical information and genome functioning so it is important to investigate the levels of DNA and RNA periodically in different tissues of the organisms undergoing stress conditions (Khanuja, 1981).

DNA (Deoxyribose nucleic acid) contents can be the index of capacity of an organism for protein synthesis in the different stress conditions affected by heavy metals or any toxic metals or pesticides. Structural changes in the DNA can be monitored using biochemical methods and usually low quantitative changes are observed on heavy metals exposure. DNA strand scission can also be sensitively monitored, and even more importantly, the specific nucleotide position cleaved can be pinpointed by biochemical methods. This methodology has been applied successfully in monitoring both the efficiency of DNA strand scission by metal complexes and the specific sites cleaved, and where the complexes are specifically bound on the helical strand, Black Marsa et. al., (1996). Genotoxicants have the ability to alter DNA and their effects may be particularly harmful as these agents can induce changes that may be passed on to future generations and have an impact on populations long after the original exposure. Environmental contaminants have been reported to induce DNA strand breaks in various mussel cells which can damage their functions Nicholson and Lam (2005). Ching et. al., (2001)
reported marked increase in strand breaks after one day exposure of *Perna viridis* to benzo[a]pyrene concentrations between 0.3 and 3 µg l⁻¹. A significant increase in DNA adduct formation has also been reported by the same author, on PAH exposure.

In some other investigations DNA strand breaks have been observed using an alkaline comet assay and the proportion of micronucleus formation in haemocytes of *P. viridis* exposed to benzo[a]pyrene, over a 12-day period Siu (2004). Zhao et. al., (1997) studied the association of arsenic induced malignant transformation with DNA hypomethylation and aberrant gene expression. Beyersmann (1994) studied that the carcinogenicity and genotoxicity of cadmium, chromium, cobalt and nickel strongly depend on their chemical ligands (speciation), which modulate them with the exception of hexavalent chromium; carcinogenic metal compounds are only weakly genotoxic. These effects are interpreted by the interference of the toxic metal ions with biochemical functions of magnesium, calcium and zinc ions. Tong Lu et. al., (2001) studied that approximately 60 genes (10%) were differentially expressed in arsenic exposed human livers as compared with those of controls, damage was also observed due to involved arsenic in the DNA of respective cells. Arsenic is known to cause DNA damage and related events, such as DNA protein cross-links, micronuclei etc (Schaumloffel and Gebel, 1998), DNA strand breaks (Lynn et. al., 1998; Liu and Jan, 2000) or alterations in DNA repair enzymes (Hartwing, 1998). Superoxide scavengers such as Cu, Zn - SOD suppress arsenic induced DNA damage.

Chatwal and Bhagi (1998) stated that for the binding of metal ions and their complex to DNA or more generally to nucleotides, there are several different coordination sites available. The metal centers can bind to the negatively charged oxygen
atoms of (poly)phosphate group or the nitrogen and oxygen atoms of purine and those containing large JL systems as ligands should also be able to inter catalane between two base pairs, possibly even in a sequence specific fashion. Lastly, coordinated ligands with, for example, amine or hydroxyl functions may form hydrogen bonds with protein acceptor components of the polynucleotides. Interactions of metal ions or metal complexes with nucleic acid play an important role:

1. In sustaining the conformation such as DNA or RNA through electrostatic effects,

2. in the nucleic acid metabolism particularly in phosphoryl transfer,

3. in the regulation, replication and transcription of genetic information,

4. For efforts directed at specific DNA cleavage with synthetic probes and

5. For metal induced mutagenesis.

Such mutations can be due to genometric distortions of the DNA through unphysiological cross linking or to the stabilization or a wrong nucleobase tautomer complex nucleic acid inter actions and their physiological consequences can thus be quite varied, even in the extensively studied platinum compounds they are far from being fully understood.

The platinum complexes act as anticancer agents by interacting with DNA and Trans platinum bind most strongly to RNA than to DNA and least strongly to proteins. When the activity was assessed as the ability to suppress the synthesis of DNA, RNA and protein, only the synthesis of DNA was suppressed.

The selectivity of platinum complexes in attacking tumor cells rather than normal cells, even through there is little or no preferential uptake of platinum in tumour cells, has
led to the suggestion that cancer cells are deficient in some DNA repair mechanisms. DNA is constantly being damaged but various repair proteins can recognize the damaged segment and cause the repair. Platinum specifically binds DNA at the minor groove.

Detmar and Andrea (1992) studied that cobalt is an essential trace element for mammalian nutrition, but also is classified as carcinogenic with the fidelity of DNA synthesis. Regarding anti and co-mutagenic mechanisms, the evidence for interference of Co (II) with DNA repair processes is known.

An excellent example of the work at the London St. Mary's Branch and the San Diego Branch, found that in a normal cell P-53 expression levels in response to stress and DNA damaging agents caused cell cycle arrest and apoptosis before the damage to the DNA of their nuclei causing oncogenesis. Bryan et al. (1986) suggested the relationship between copper and nucleo-proteins and copper related changes in DNA cleavage have been reported by Ehrenfield et. al., (1987).

A severe copper depletion has been recorded because of a major burn (Bryant et al., 2003) and production of several organics as Ceruloplasmin (Danks et al, 1986) and Cytochrome C-552 (Merchant and Bogorad, 1987b) has been demonstrated to be genetically controlled but the genetic expression is dictated by copper availability to the cells. Even certain nucleoproteins are copper rich (Bryan et al., 1985) although it is not known, if the metals complexes to protein, to the nucleic acid, or exists in a protein copper nucleic acid complex (Bryan et al., 1986). Basile and Barton (1987) presented the design of a double stranded DNA cleavage agent with two-polyamine metal binding arms, which they suggested might be able to deliver metal activated chemistry to one or
both DNA strands. Black et al. (1996) found significant DNA strand breakage in different tissues from *Anodonta grandis* exposed to toxicant.

Pesticide is known to cause DNA damage and related events, such as DNA protein cross-links, micronuclei etc. (Schaumloffel and Gebel, 1998), DNA strand breaks (Lynn et al, 1998; Liu and Jan, 2000), or alterations in DNA repair enzymes (Hartwing, 1998). Supper oxide scavengers such as Cu, Zn - SOD suppress arsenic induced DNA damage (Hartwing, 1998; Lynn et al, 1998; Liu and Jan, 2000).

Tong Lu et al. (2001) studied that approximately 60 genes (10%) were differentially expressed in arsenic exposed human livers as compared with those of controls, damage was also observed due to involved arsenic in the DNA of respective cells.

Low-dose exposures to pesticide are not likely to cause cancer in humans. Data on effects related to mutation formation (Changes in DNA) indicates that pesticide could increase frequencies of mutation in human eggs and sperm.

Excess pesticide, produces DNA damage when introduced into as cite tumors. Zhao et al. (1997) studied the association of arsenic induced malignant transformation with DNA hypomethylation and aberrant gene expression. Beyersmann (1994) studied that the carcinogenicity and genotoxicity of cadmium, chromium, cobalt and nickel strongly depend on their chemical ligands (speciation), which modulate them with the exception of hexavalent chromium; carcinogenic metal compounds are only weakly genotoxic. These effects are interpreted by the interference of the toxic metal ions with biochemical functions of magnesium, calcium and zinc ions.
Considering the role of DNA and paucity of information on its level in the tissues of fresh water bivalve, and considering the impact of pesticides on DNA content, it was planned to study the changes in the DNA content during acute and chronic exposure to pesticides indoxacarb and thiamethoxam from the mantle, foot, gill, digestive gland and whole body of bivalve *Parreysia cylindrica*.

**RNA:**

In order to be expressed as protein, the genetic information must be carried to the protein-synthesizing machinery of the cell, which is in the cell's cytoplasm. One form of RNA mediates this process. RNA is similar to DNA, but contains the sugar ribose instead of deoxyribose and the base uracil (U) instead of thymine. To initiate the process of information transfer, one strand of the double-stranded DNA chain serves as a template for the synthesis of a single strand of RNA that is complementary to the DNA strand (e.g., the DNA sequence AGTC … will specify an RNA sequence UCAG …). This process is called transcription and is mediated by enzymes. RNA is the polymer of the ribonucleotides held together by 3’, 5’ Phosphodiester Bridge. RNA has certain similarities with DNA structure. The distinct types of the RNA with their cellular compositions are - messenger RNA 5-10%, Transfer RNA 10-20%, and ribosomal RNA 50-80 %. RNA is capable of carrying out a multitude of diverse biological functions. Many biologically active RNA have to adopt intricate 3D structures that rival protein structures in their complexity to be functional in a cellular environment.

Similar decreased amount of RNA levels was observed by Patil and Lomte (1989) in *Mythimna (Pseudoletia) seperata* and by Chaudhari *et. al.*, (1993) in *Thiara lineata* under different toxic stress. The cellular degradation, rapid histolysis and
decreased rate of protein synthesis are the possible reasons. The role of RNA is to help protein synthesis in the cytoplasm hence depletion of RNA level also resulted decreased rate of protein synthesis (Rao et al., 1990). Ester Saball et al. (2000) observed the total tissue m-RNA of liver and kidneys of control and HgCl₂ treated rats. Tong Lu et al. (2001) observed that 10% genes, mostly related to cell cycle regulation, apoptosis, DNA damage response etc. were differentially expressed in the form of RNA and such abnormal RNA are vulnerable to RNA are attack. Ermachenko et. al., (1987) observed reduction in weight of testis and prostate, increased RNA concentration in testis, solerosis of leyding cells and increase in the number of normal spermatozoa after inhalation of CuCl₂ for three months. Rao et al., (1998) studied the RNA levels in various tissues of freshwater crab Barytelphusa cunicularis when exposed to Fluoride. Ester Saball et al. (2000) observed the total tissue m-RNA of liver and kidneys of control and HgCl₂ treated rats. Tong Lu et. al., (2001) observed that 10% genes, mostly related to cell cycle regulation, apoptosis, DNA damage response etc. were differentially expressed in the form of RNA and such abnormal RNA are vulnerable to RNA are attack. Heavy metals may interact with RNA polymerases which causes adverse effect. RNA polymerase must bind site specifically to its DNA template, binds its nucleotide and primer substrate, and form new phosphodiester bond in elongating the growing RNA. Zinc ion appears to be essential for the function of both RNA polymerase and DNA topoisomerase, (Sahasrabudhe et. al., 1995). In any tissues, toxic influences exert their effect first at the molecular and biochemical level (Robbins and Angel, 1976), hence alteration in normal biochemical parameters serve as the earliest indicators of toxic effect on tissues. These have been referred to as reliable tools for evaluating the extent of hazard of any
chemicals much before any gross signs become apparent (Johnson and Heijnen, 2001)).

In present study, freshwater bivalve, *Parreysia cylindrica* is used as test model to detect the drastic effect of indoxacarb and thiamethoxam. The biochemical contents such as Protein, Ascorbic acid, DNA and RNA are studied as the indicators from different tissues, i.e. whole body, mantle gill, hepatopancreas, foot and gonad. Reduction of toxicant reduces the stress and hence reduces level of stress effects.

Pesticide also interacts with RNA polymerases. Severe effects are expressed as such in DNA metal binding. RNA polymerase must bind site specifically to its DNA template, binds its nucleotide and primer substrates, and form a new phosphodiester bond in elongating the growing RNA. Zinc ion appears to be essential to the functioning to RNA polymerases and DNA topoisomerases, (Giedroc and Coleman, 1989).

Eukaryotic RNA polymerases I, II and III are involved in the synthesis of ribosomal, messenger and transfer RNAs, respectively. The DNA dependent RNA polymerases I (Falchuk *et. al.*, 1977), II (Falchuk *et. al.*, 1976) and III (Wandzilak and Benson, 1977) of the unicellular eukaryote *Euglena gracilis* have all been showed to be zinc metallo enzyme, each binding about 2 gram atoms of zinc.

The role of RNA is to help protein synthesis in the cytoplasm hence depletion of RNA level also resulted decreased rate of protein synthesis (Rao *et. al.*, 1990). Similar decreased amount of RNA levels was observed by Asfia and Vasantha (1988) in *Clarius batracus*, by Patil and Lomte (1989) in *Mythimna (Pseudoletia) seperata* and by Chaudhari *et al*. (1993) in *Thiara lineata* under different toxic stress. The cellular degradation, rapid histolysis and decreased rate of protein synthesis are the possible reasons.
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Very little information is available on the variation in protein, free amino acid, ascorbic acid, DNA and RNA content in the fresh water bivalves exposed to pesticides. The present study is thus undertaken to find out the change in biochemical components of fresh water bivalves, Parreysia cylindrica after acute and chronic exposure to indoxacarb and thiamethoxam.
MATERIALS AND METHODS

The fresh water bivalve *Parreysia cylindrica* were collected from the Jamda dam which is nearly 30 Kms away from Chalisgaon, Dist. Jalgaon (M.S). After collection, the bivalves were acclimatized in the laboratory condition at room temperature for 4-6 days. The active acclimatized bivalves of approximately same size were selected for experimentation.

Before starting the experiment, these bivalves were divided into five groups such as A, B, C, D and E.

1. ‘A’ group of bivalves were maintained as control.
2. ‘B’ group of bivalves were exposed to sub lethal dose (0.3905 ppm LC$_{50}/2$ of 96 hrs) of indoxacarb upto 96 hours
3. ‘C’ group of bivalves were exposed to sub lethal dose (14.2114 ppm LC$_{50}/2$ of 96 hrs) of thiamethoxam, upto 96 hours
4. ‘D’ group of bivalves were exposed to chronic dose (0.07811 ppm LC$_{50}/10$ of 96 hrs) of indoxacarb, upto 21 days
5. ‘E’ group of bivalves were exposed to chronic dose (2.8422 ppm LC$_{50}/10$ of 96 hrs) of thiamethoxam, upto 21 days

The control and experimental bivalves of A, B and C groups were dissected after 24, 48, 72 and 96 hours and animals from A,D,E group of chronic treatment after 7 days ,14 days and 21 days. Their mantle, gills, foot, digestive glands and whole body were removed. These tissues were dried in oven at 75 $^\circ$C to 80 $^\circ$C till constant weight was obtained and blended into dry powder. These powders were used for the estimation of
various biochemical components (protein, amino acid, ascorbic acid, DNA and RNA). The methods of estimation were as follows -

**Protein estimation:**

Protein content of the tissues was estimated by Lowry’s method (Lowry et al., 1951). 10 mg. of dry powder was homogenized in small amount of 10% TCA and the homogenate was diluted to 10 ml by 10% TCA. Then it was centrifuged at 3000 rpm for 15 minutes. The supernatant was removed which was used for ascorbic acid estimation. The protein precipitate at the bottom of centrifuged tubes was dissolved in 10 ml 1.0 N NaOH solution. 0.1 ml of this solution of each powder and 0.9 ml distilled water were taken in three test tubes containing 4.0 ml freshly prepared Lowry’s ‘C’. After adding 0.5 ml Folin’s – phenol reagent, the test tubes were incubated in dark at 37°C for 30 minutes. The O. D. of blue colour developed was read at 530 nm. The blank was prepared in same way without dissolved protein precipitate.

The protein content in different tissues was calculated referring to standard graph value and it was expressed in terms of mg protein/100 mg of dry tissue. The Bovine serum albumen was used as a standard.

**Free Amino-acids:**

Free amino acids were estimated by the method of Moore and stein(1954)100mg of tissue powder was added to 5 ml of 10% TCA and centrifugated at 3000 rpm for 15 minutes to 0.5 ml of the supernatant, 2ml of Ninhydrin reagent was added and kept in boiling water bath for 6 minutes and cooled immediately. The purple colour developed was recorded at 530 nm, using the mixture of 2 ml ninhydrin reagent and 5 ml TCA
solution as a blank. The concentration of free amino acids was calculated by using the standard graph obtained by various concentrations of the mixture of glutamic acid and glycine.

**Ascorbic acid estimation:**

Ascorbic acid estimation was carried out by the method of Roe (1967). 1.0 ml supernatant was taken in test tubes from the homogenate which was already centrifuged for protein estimation. In these test tubes 0.25 ml aliquot of hydrazine reagent was added. The reaction mixture was kept in boiling water bath for 15 minutes. It was cooled and 3.0 ml ice cold 85% H2SO4 was added drop wise with constant stirring. The reaction mixture was kept at room temperature for 30 minutes. O. D. was read at 530 nm.

Ascorbic acid was used as a standard. Amount of ascorbic acid in different tissues was calculated from standard graph values. It was expressed as mg of ascorbic acid per 100 mg of dry tissue.

**DNA estimation:**

DNA content of the tissue was measured by using Diphenylamine method of Burton (1956). 10mg of dry tissue powder was homogenized by adding 10ml distilled water. Then it was centrifuged at 3000 rpm for 10 minutes. The supernatant containing DNA was removed. After that 1ml supernatant was taken and 3ml diphenylamine reagent was added. Then the solution in the test tube was placed in boiling water bath for 10 minutes. After boiling the solution in the test tube was allowed to cool. Then the optical density of the DNA was read at 595 nm filter.
RNA estimation:

RNA content of the tissue was measured by following Orcinol method of Volkin and Cohn (1954). 10mg of dry tissue powder was homogenized by adding 10ml distilled water. Then it was centrifuged at 3000rpm for 10 minutes. The supernatant containing RNA was removed. After that 1ml supernatant was taken and 3ml Orcinol reagent was added. Then the solution in the test tube was placed in boiling water bath for 15 minutes. After boiling the solution in the test tube was allowed to cool. Then the optical density of the RNA was read at 665 nm filter.

Each observation was confirmed by taking at least three replicates. The differences in control and experimental animal group was tested for significance by using student ‘t’ test (Baily,1965) and the percentage decreases or increases over control were calculated for each value.
OBSERVATIONS AND RESULTS

Changes in protein, amino acid, ascorbic acid RNA, DNA and free amino acid contents in gills, gonads and digestive glands of freshwater bivalve, Parreysia cylindrica after exposure to acute and chronic concentrations of pesticides, indoxacarb and thiamethoxam for 24 and 96 hours and 7 and 21 days are summarized in table No.3.1.1 to 3.1.10

Protein content in mantle:

The protein content in mantle of bivalves in control group at 24 hours was 48.25 mg/100 mg of dry tissue for 96 hours it was 47.69 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the protein content in mantle was 40.36 mg/100 mg of dry tissue after 96 hours exposure it was 38.56 mg/100 mg of dry tissue.

Protein content in mantle of bivalves exposed to acute concentration of thiamethoxam (14.2114ppm) was 43.36 mg/100 mg of dry tissue after 24 hours exposure and it was 36.26 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the protein contents after exposure to the pesticides.

Protein content in foot:

The protein content in foot of bivalves in control group at 24 hours was 68.91 mg/100 mg of dry tissue for 96 hours it was 68.212 mg/100 mg of dry tissue.
In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the protein content in mantle was 57.91 mg/100 mg of dry tissue after 96 hours exposure it was 50.91 mg/100 mg of dry tissue.

Protein content in foot of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 60.26 mg/100 mg of dry tissue after 24 hours exposure and it was 53.01 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the protein contents after exposure to the pesticides.

**Protein content in gills:**

The protein content in gills of bivalves in control group at 24 hours was 54.11 mg/100 mg of dry tissue for 96 hours it was 53.74 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the protein content in gills was 43.86 mg/100 mg of dry tissue after 96 hours exposure it was 38.26 mg/100 mg of dry tissue.

Protein content in gills of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 48.05 mg/100 mg of dry tissue after 24 hours exposure and it was 40.12 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the protein contents after exposure to the pesticides.

**Protein content in digestive glands:**

Digestive glands of bivalves in control group showed protein content as 51.31 percent of dry tissue for 24 hours and 51.216 percent of dry tissue for 96 hours.
In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), the protein content in digestive glands was 38.12 mg/100 mg of dry tissue after 24 hours exposure and 29.89 mg/100 mg of dry tissue after 96 hours exposure.

Protein content in digestive glands of bivalves exposed to acute concentration of thiamethoxam (14.2114) was 42.89 mg/100 mg of dry tissue after 24 hours exposure and it was 35.25 mg/100 mg of dry tissue after 96 hours exposure.

**Protein content in whole soft body:**

The protein content in whole soft body of bivalves in control group at 24 hours was 58.21 mg/100 mg of dry tissue for 96 hours it was 58.196 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the protein content in whole soft body was 42.46 mg/100 mg of dry tissue after 96 hours exposure it was 38.612mg/100 mg of dry tissue.

Protein content in whole soft body of bivalves exposed to acute concentration of thiamethoxam (14.2114ppm) was 49.965 mg/100 mg of dry tissue after 24 hours exposure and it was 45.316 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the protein contents after exposure to the pesticides.

**Protein content in mantle:**

The protein content in mantle of bivalves in control group at 7 days was 49.25 mg/100 mg of dry tissue for 21days it was 48.69 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7days the protein content in mantle was 39.66 mg/100 mg of dry tissue after 21 days exposure it was 31.58 mg/100 mg of dry tissue.
Protein content in mantle of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 41.50 mg/100 mg of dry tissue after 7 days exposure and it was 34.21 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the protein contents after exposure to the pesticides.

**Protein content in foot:**

The protein content in foot of bivalves in control group at 7 days was 69.11 mg/100 mg of dry tissue for 21 days it was 68.612 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the protein content in foot was 56.91 mg/100 mg of dry tissue after 21 days exposure it was 45.12 mg/100 mg of dry tissue.

Protein content in foot of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 59.75 mg/100 mg of dry tissue after 7 days exposure and it was 49.561 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the protein contents after exposure to the pesticides.

**Protein content in gills:**

The protein content in gills of bivalves in control group at 7 days was 54.91 mg/100 mg of dry tissue for 21 days it was 54.34 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the protein content in gills was 42.076 mg/100 mg of dry tissue after 21 days exposure it was 33.619 mg/100 mg of dry tissue.

Protein content in gills of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 45.61 mg/100 mg of dry tissue after 7 days exposure
and it was 37.076 mg/ 100 mg of dry tissue after 21 days exposure. There was significant variation in the protein contents after exposure to the pesticides.

**Protein content in digestive gland:**

The protein content in digestive gland of bivalves in control group at 7 days was 51.51 mg/ 100 mg of dry tissue for 21 days it was 51.216 mg/ 100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.0.07811 ppm), at 7 days the protein content in digestive gland was 37.06 mg/ 100 mg of dry tissue after 21 days exposure it was 28.65 mg/ 100 mg of dry tissue.

Protein content in digestive of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 41.36 mg/ 100 mg of dry tissue after 7 days exposure and it was 33.26 mg/ 100 mg of dry tissue after 21 days exposure. There was significant variation in the protein contents after exposure to the pesticides.

**Protein content in whole soft body:**

The protein content in whole soft body of bivalves in control group at 7 days was 59.23 mg/ 100 mg of dry tissue for 21 days it was 57.196 mg/ 100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.0.07811 ppm), at 7 days the protein content in whole soft body was 42.216 mg/ 100 mg of dry tissue after 21 days exposure it was 36.161 mg/ 100 mg of dry tissue.

Protein content in whole soft body of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 46.965 mg/ 100 mg of dry tissue after 7 days exposure and it was 42.02 mg/ 100 mg of dry tissue after 21 days exposure. There was significant variation in the protein contents after exposure to the pesticides.
Amino acid content in mantle:

The amino acid content in mantle of bivalves in control group at 24 hours was 62.101 mg/ gm of dry tissue for 96 hours it was 61.878 mg/ gm of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the amino acid content in mantle was 73.082 mg/ gm of dry tissue after 96 hours exposure it was 77.572 mg/ gm of dry tissue.

Amino acid content in mantle of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 68.3705 mg/ gm of dry tissue after 24 hours exposure and it was 75.572 mg/ gm of dry tissue after 96 hours exposure. There was significant variation in the amino acid contents after exposure to the pesticides.

Amino acid content in foot:

The amino acid content in foot of bivalves in control group at 24 hours was 67.517 mg/ gm of dry tissue for 96 hours it was 67.146 mg/ gm of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the amino acid content in foot was 77.8356 mg/ gm of dry tissue after 96 hours exposure it was 84.5265 mg/ gm of dry tissue.

Amino acid content in foot of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 76.0805 mg/ gm of dry tissue after 24 hours exposure and it was 82.572 mg/ gm of dry tissue after 96 hours exposure. There was significant variation in the amino acid contents after exposure to the pesticides.
Amino acid content in gill:

The amino acid content in gill of bivalves in control group at 24 hours was 71.642 mg/ gm of dry tissue for 96 hours it was 70.796 mg/ gm of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the amino acid content in gill was 84.987 mg/ gm of dry tissue after 96 hours exposure it was 92.0325 mg/ gm of dry tissue.

Amino acid content in gill of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 80.3965 mg/ gm of dry tissue after 24 hours exposure and it was 87.121 mg/ gm of dry tissue after 96 hours exposure. There was significant variation in the amino acid contents after exposure to the pesticides.

Amino acid content in digestive gland:

The amino acid content in digestive gland of bivalves in control group at 24 hours was 126.750 mg/ gm of dry tissue for 96 hours it was 124.345 mg/ gm of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the amino acid content in gill was 158.98 mg/ gm of dry tissue after 96 hours exposure it was 178.9596 mg/ gm of dry tissue.

Amino acid content in gill of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 148.1426 mg/ gm of dry tissue after 24 hours exposure and it was 163.386 mg/ gm of dry tissue after 96 hours exposure. There was significant variation in the amino acid contents after exposure to the pesticides.
Amino acid content in whole soft body:

The amino acid content in whole soft body of bivalves in control group at 24 hours was 65.086 mg/gm of dry tissue for 96 hours it was 64.245 mg/gm of dry tissue. In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the amino acid content in gill was 83.1254 mg/gm of dry tissue after 96 hours exposure it was 87.1472 mg/gm of dry tissue.

Amino acid content in gill of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 74.523 mg/gm of dry tissue after 24 hours exposure and it was 78.5667 mg/gm of dry tissue after 96 hours exposure. There was significant variation in the amino acid contents after exposure to the pesticides.

Amino acid content in mantle:

The amino acid content in mantle of bivalves in control group at 7 day was 61.5743 mg/gm of dry tissue for 21 day it was 58.3547 mg/gm of dry tissue. In the bivalves exposed to chronic concentration of indoxacarb ((0.07811 ppm), at 7 days the amino acid content in mantle was 73.5874 mg/gm of dry tissue after 21 days exposure it was 79.3578 mg/gm of dry tissue.

Amino acid content in mantle of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 71.1478 mg/gm of dry tissue after 7 days exposure and it was 75.3578 mg/gm of dry tissue after 21 days exposure. There was significant variation in the amino acid contents after exposure to the pesticides.
**Amino acid content in foot:**

The amino acid content in foot of bivalves in control group at 7 day was 65.0125 mg/ gm of dry tissue for 21 day it was 62.3214 mg/ gm of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb ((0.07811 ppm), at 7 days the amino acid content in foot was 76.1689 mg/ gm of dry tissue after 21 days exposure it was 83.8356 mg/ gm of dry tissue.

Amino acid content in mantle of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 74.0147 mg/ gm of dry tissue after 7 days exposure and it was 79.3478 mg/ gm of dry tissue after 21 days exposure. There was significant variation in the amino acid contents after exposure to the pesticides.

**Amino acid content in gill:**

The amino acid content in gill of bivalves in control group at 7 day was 69.0231 mg/ gm of dry tissue for 21 day it was 68.0124 mg/ gm of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb ((0.07811 ppm), at 7 days the amino acid content in gill was 84.9795 mg/ gm of dry tissue after 21 days exposure it was 94.1478 mg/ gm of dry tissue.

Amino acid content in gill of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 80.5879 mg/ gm of dry tissue after 7 days exposure and it was 89.3689 mg/ gm of dry tissue after 21 days exposure. There was significant variation in the amino acid contents after exposure to the pesticides.
**Amino acid content in digestive gland:**

The amino acid content in digestive gland of bivalves in control group at 7 days was 124.8752 mg/gm of dry tissue for 21 day it was 123.5487 mg/gm of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the amino acid content in digestive gland was 160.8147 mg/gm of dry tissue after 21 days exposure it was 179.0235 mg/gm of dry tissue.

Amino acid content in digestive gland of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 149.2548 mg/gm of dry tissue after 7 days exposure and it was 167.358 mg/gm of dry tissue after 21 days exposure. There was significant variation in the amino acid contents after exposure to the pesticides.

**Amino acid content in whole soft body:**

The amino acid content in whole soft body of bivalves in control group at 7 day was 63.2147 mg/gm of dry tissue for 21 day it was 62.0874 mg/gm of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the amino acid content in whole soft body was 78.2345 mg/gm of dry tissue after 21 days exposure it was 84.8151 mg/gm of dry tissue.

Amino acid content in whole soft body of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 75.8578 mg/gm of dry tissue after 7 days exposure and it was 78.5421 mg/gm of dry tissue after 21 days exposure. There was significant variation in the amino acid contents after exposure to the pesticides.
L-Ascorbic content in mantle:

The L-Ascorbic acid content in mantle of bivalves in control group at 24 hours was 0.789 mg/100 mg of dry tissue for 96 hours it was 0.751 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the Ascorbic acid content in mantle was 0.665 mg/100 mg of dry tissue after 96 hours exposure it was 0.538 mg/100 mg of dry tissue.

Ascorbic acid content in mantle of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 0.699 mg/100 mg of dry tissue after 24 hours exposure and it was 0.584 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the ascorbic contents after exposure to the pesticides.

L-Ascorbic content in foot:

The L-Ascorbic acid content in foot of bivalves in control group at 24 hours was 0.875 mg/100 mg of dry tissue for 96 hours it was 0.845 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the Ascorbic acid content in foot was 0.745 mg/100 mg of dry tissue after 96 hours exposure it was 0.652 mg/100 mg of dry tissue.

Ascorbic acid content in foot of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 0.789 mg/100 mg of dry tissue after 24 hours exposure and it was 0.692 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the ascorbic contents after exposure to the pesticides.
L-Ascorbic content in gills:

The L-Ascorbic acid content in gills of bivalves in control group at 24 hours was 0.749 mg/100 mg of dry tissue for 96 hours it was 0.723 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the Ascorbic acid content in gills was 0.609 mg/100 mg of dry tissue after 96 hours exposure it was 0.509 mg/100 mg of dry tissue.

Ascorbic acid content in gills of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 0.667 mg/100 mg of dry tissue after 24 hours exposure and it was 0.553 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the ascorbic contents after exposure to the pesticides.

L-Ascorbic content in digestive gland:

The L-Ascorbic acid content in digestive gland of bivalves in control group at 24 hours was 0.889 mg/100 mg of dry tissue for 96 hours it was 0.874 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the Ascorbic acid content in digestive gland was 0.645 mg/100 mg of dry tissue after 96 hours exposure it was 0.564 mg/100 mg of dry tissue.

Ascorbic acid content in digestive gland of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 0.731 mg/100 mg of dry tissue after 24 hours exposure and it was 0.623 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the ascorbic contents after exposure to the pesticides.
**L-Ascorbic content in whole soft body:**

The L-Ascorbic acid content in whole soft body of bivalves in control group at 24 hours was 0.817mg/ 100 mg of dry tissue for 96 hours it was 0.811 mg/ 100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the Ascorbic acid content in whole soft body was 0.692 mg/ 100 mg of dry tissue after 96 hours exposure it was 0.574 mg/ 100 mg of dry tissue.

Ascorbic acid content in whole soft body of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 0.712 mg/ 100 mg of dry tissue after 24 hours exposure and it was 0.622 mg/ 100 mg of dry tissue after 96 hours exposure. There was significant variation in the ascorbic contents after exposure to the pesticides.

**L-Ascorbic content in mantle:**

The L-Ascorbic acid content in mantle of bivalves in control group at 7 days was 0.799 mg/ 100 mg of dry tissue for 21 days it was 0.756 mg/ 100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the ascorbic acid content in mantle was 0.640 mg/ 100 mg of dry tissue after 21 days exposure it was 0.469 mg/ 100 mg of dry tissue.

Ascorbic acid content in mantle of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 0.682 mg/ 100 mg of dry tissue after 7 days exposure and it was 0.546 mg/ 100 mg of dry tissue after 21 days exposure. There was significant variation in the ascorbic contents after exposure to the pesticides.
L-Ascorbic content in foot:

The L-Ascorbic acid content in foot of bivalves in control group at 7 days was 0.885 mg/100 mg of dry tissue for 21 days it was 0.845 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the Ascorbic acid content in foot was 0.716 mg/100 mg of dry tissue after 21 days exposure it was 0.559 mg/100 mg of dry tissue.

Ascorbic acid content in foot of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 0.770 mg/100 mg of dry tissue after 7 days exposure and it was 0.650 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the ascorbic contents after exposure to the pesticides.

L-Ascorbic content in gill:

The L-Ascorbic acid content in gill of bivalves in control group at 7 days was 0.789 mg/100 mg of dry tissue for 21 days it was 0.769 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the Ascorbic acid content in gill was 0.601 mg/100 mg of dry tissue after 21 days exposure it was 0.468 mg/100 mg of dry tissue.

Ascorbic acid content in gill of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 0.645 mg/100 mg of dry tissue after 7 days exposure and it was 0.547 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the ascorbic contents after exposure to the pesticides.
L-Ascorbic content in digestive gland:

The L-Ascorbic acid content in digestive gland of bivalves in control group at 7 days was 0.895 mg/100 mg of dry tissue for 21 days it was 0.871 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the Ascorbic acid content in digestive gland was 0.635 mg/100 mg of dry tissue after 21 days exposure it was 0.494 mg/100 mg of dry tissue.

Ascorbic acid content in digestive gland of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 0.739 mg/100 mg of dry tissue after 7 days exposure and it was 0.573 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the ascorbic contents after exposure to the pesticides.

L-Ascorbic content in whole soft body:

The L-Ascorbic acid content in whole soft body of bivalves in control group at 7 days was 0.832 mg/100 mg of dry tissue for 21 days it was 0.811 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the ascorbic acid content in whole soft body was 0.623 mg/100 mg of dry tissue after 21 days exposure it was 0.532 mg/100 mg of dry tissue.

Ascorbic acid content in whole soft body of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 0.686 mg/100 mg of dry tissue after 7 days exposure and it was 0.615 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the ascorbic contents after exposure to the pesticides.
DNA content in mantle:

The DNA content in mantle of bivalves in control group at 24 hours was 1.345 mg/ 100 mg of dry tissue for 96 hours it was 1.340 mg/ 100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the DNA content in mantle was 1.200 mg/ 100 mg of dry tissue after 96 hours exposure it was 1.017 mg/ 100 mg of dry tissue.

DNA content in mantle of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 1.276 mg/ 100 mg of dry tissue after 24 hours exposure and it was 1.056 mg/ 100 mg of dry tissue after 96 hours exposure. There was significant variation in the DNA contents after exposure to the pesticides.

DNA content in foot:

The DNA content in foot of bivalves in control group at 24 hours was 2.577 mg/ 100 mg of dry tissue for 96 hours it was 2.549 mg/ 100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the DNA content in foot was 2.269 mg/ 100 mg of dry tissue after 96 hours exposure it was 2.021 mg/ 100 mg of dry tissue.

DNA content in foot of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 2.343 mg/ 100 mg of dry tissue after 24 hours exposure and it was 2.045 mg/ 100 mg of dry tissue after 96 hours exposure. There was significant variation in the DNA contents after exposure to the pesticides.
**DNA content in gill:**

The DNA content in gill of bivalves in control group at 24 hours was 1.238 mg/100 mg of dry tissue for 96 hours it was 1.19 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the DNA content in gill was 1.035 mg/100 mg of dry tissue after 96 hours exposure it was 0.857 mg/100 mg of dry tissue.

DNA content in gill of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 1.11 mg/100 mg of dry tissue after 24 hours exposure and it was 0.898 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the DNA contents after exposure to the pesticides.

**DNA content in digestive gland:**

The DNA content in digestive gland of bivalves in control group at 24 hours was 2.318 mg/100 mg of dry tissue for 96 hours it was 2.298 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the DNA content in digestive gland was 1.683 mg/100 mg of dry tissue after 96 hours exposure it was 1.458 mg/100 mg of dry tissue.

DNA content in digestive gland of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 1.835 mg/100 mg of dry tissue after 24 hours exposure and it was 1.59 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the DNA contents after exposure to the pesticides.
DNA content in whole soft body:

The DNA content in whole soft body of bivalves in control group at 24 hours was 2.371 mg/ 100 mg of dry tissue for 96 hours it was 2.365 mg/ 100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the DNA content in whole soft body was 2.105 mg/ 100 mg of dry tissue after 96 hours exposure it was 1.642 mg/ 100 mg of dry tissue.

DNA content in whole soft body of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 2.212 mg/ 100 mg of dry tissue after 24 hours exposure and it was 1.844 mg/ 100 mg of dry tissue after 96 hours exposure. There was significant variation in the DNA contents after exposure to the pesticides.

DNA content in mantle:

The DNA content in mantle of bivalves in control group at 7days was 1.455 mg/ 100 mg of dry tissue for 21 days it was 1.421 mg/ 100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.0.07811 ppm), at 7days the DNA content in mantle was 1.197 mg/ 100 mg of dry tissue after 21 days exposure it was 0.977 mg/ 100 mg of dry tissue.

DNA content in mantle of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 1.250 mg/ 100 mg of dry tissue after 7 days exposure and it was 1.043 mg/ 100 mg of dry tissue after 21 days exposure. There was significant variation in the DNA contents after exposure to the pesticides.
DNA content in foot:

The DNA content in foot of bivalves in control group at 7 days was 2.612 mg/100 mg of dry tissue for 21 days it was 2.598 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.0.07811 ppm), at 7 days the DNA content in foot was 2.209 mg/100 mg of dry tissue after 21 days exposure it was 1.898 mg/100 mg of dry tissue.

DNA content in foot of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 2.245 mg/100 mg of dry tissue after 7 days exposure and it was 2.058 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the DNA contents after exposure to the pesticides.

DNA content in gill:

The DNA content in gill of bivalves in control group at 7 days was 1.249 mg/100 mg of dry tissue for 21 days it was 1.238 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.0.07811 ppm), at 7 days the DNA content in gill was 0.937 mg/100 mg of dry tissue after 21 days exposure it was 0.764 mg/100 mg of dry tissue.

DNA content in gill of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 1.025 mg/100 mg of dry tissue after 7 days exposure and it was 0.887 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the DNA contents after exposure to the pesticides.
DNA content in digestive gland:

The DNA content in digestive gland of bivalves in control group at 7days was 2.297 mg/100 mg of dry tissue for 21 days it was 2.256 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.007811 ppm), at 7 days the DNA content in digestive gland was 1.420 mg/100 mg of dry tissue after 21 days exposure it was 0.948 mg/100 mg of dry tissue.

DNA content in digestive gland of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 1.487 mg/100 mg of dry tissue after 7 days exposure and it was 1.197 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the DNA contents after exposure to the pesticides.

DNA content in whole soft body:

The DNA content in whole soft body of bivalves in control group at 7 days was 2.412 mg/100 mg of dry tissue for 21 days it was 2.398 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.007811 ppm), at 7 days the DNA content in whole soft body was 2.004 mg/100 mg of dry tissue after 21 days exposure it was 1.665 mg/100 mg of dry tissue.

DNA content in whole soft body of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 2.034 mg/100 mg of dry tissue after 7 days exposure and it was 1.795 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the DNA contents after exposure to the pesticides.
RNA content in mantle:

The RNA content in mantle of bivalves in control group at 24 hours was 4.895 mg/ 100 mg of dry tissue for 96 hours it was 4.781 mg/ 100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the RNA content in mantle was 4.056 mg/ 100 mg of dry tissue after 96 hours exposure it was 3.545 mg/ 100 mg of dry tissue.

RNA content in mantle of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 4.352 mg/ 100 mg of dry tissue after 24 hours exposure and it was 3.889 mg/ 100 mg of dry tissue after 96 hours exposure. There was significant variation in the RNA contents after exposure to the pesticides.

RNA content in foot:

The RNA content in foot of bivalves in control group at 24 hours was 5.671 mg/ 100 mg of dry tissue for 96 hours it was 5.612 mg/ 100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the RNA content in foot was 4.875 mg/ 100 mg of dry tissue after 96 hours exposure it was 4.256 mg/ 100 mg of dry tissue.

RNA content in foot of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 5.123 mg/ 100 mg of dry tissue after 24 hours exposure and it was 4.697 mg/ 100 mg of dry tissue after 96 hours exposure. There was significant variation in the RNA contents after exposure to the pesticides.
RNA content in gill:

The RNA content in gill of bivalves in control group at 24 hours was 6.136 mg/100 mg of dry tissue for 96 hours it was 6.111 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the RNA content in gill was 5.069 mg/100 mg of dry tissue after 96 hours exposure it was 4.168 mg/100 mg of dry tissue.

RNA content in gill of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 5.694 mg/100 mg of dry tissue after 24 hours exposure and it was 4.786 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the RNA contents after exposure to the pesticides.

RNA content in digestive gland:

The RNA content in digestive gland of bivalves in control group at 24 hours was 8.642 mg/100 mg of dry tissue for 96 hours it was 8.581 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the RNA content in digestive gland was 6.15 mg/100 mg of dry tissue after 96 hours exposure it was 4.645 mg/100 mg of dry tissue.

RNA content in digestive gland of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 6.545 mg/100 mg of dry tissue after 24 hours exposure and it was 5.151 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the RNA contents after exposure to the pesticides.
RNA content in whole soft body:

The RNA content in whole soft body of bivalves in control group at 24 hours was 6.878 mg/100 mg of dry tissue for 96 hours it was 6.871 mg/100 mg of dry tissue.

In the bivalves exposed to acute concentration of indoxacarb (0.3905 ppm), at 24 hours the RNA content in whole soft body was 5.843 mg/100 mg of dry tissue after 96 hours exposure it was 4.619 mg/100 mg of dry tissue.

RNA content in whole soft body of bivalves exposed to acute concentration of thiamethoxam (14.2114 ppm) was 6.112 mg/100 mg of dry tissue after 24 hours exposure and it was 5.132 mg/100 mg of dry tissue after 96 hours exposure. There was significant variation in the RNA contents after exposure to the pesticides.

RNA content in mantle:

The RNA content in mantle of bivalves in control group at 7 days was 4.879 mg/100 mg of dry tissue for 21 days it was 4.759 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the RNA content in mantle was 3.884 mg/100 mg of dry tissue after 21 days exposure it was 3.092 mg/100 mg of dry tissue.

RNA content in mantle of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 4.124 mg/100 mg of dry tissue after 7 days exposure and it was 3.451 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the RNA contents after exposure to the pesticides.
RNA content in foot:

The RNA content in foot of bivalves in control group at 7 days was 5.665 mg/100 mg of dry tissue for 21 days it was 5.423 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the RNA content in foot was 4.697 mg/100 mg of dry tissue after 21 days exposure it was 3.621 mg/100 mg of dry tissue.

RNA content in foot of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 4.851 mg/100 mg of dry tissue after 7 days exposure and it was 4.135 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the RNA contents after exposure to the pesticides.

RNA content in gill:

The RNA content in gill of bivalves in control group at 7 days was 6.113 mg/100 mg of dry tissue for 21 days it was 6.011 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the RNA content in gill was 4.825 mg/100 mg of dry tissue after 21 days exposure it was 3.009 mg/100 mg of dry tissue.

RNA content in gill of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 5.245 mg/100 mg of dry tissue after 7 days exposure and it was 4.25 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the RNA contents after exposure to the pesticides.
RNA content in digestive gland:

The RNA content in digestive gland of bivalves in control group at 7 days was 8.594 mg/100 mg of dry tissue for 21 days it was 8.486 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the RNA content in digestive gland was 5.852 mg/100 mg of dry tissue after 21 days exposure it was 4.035 mg/100 mg of dry tissue.

RNA content in digestive gland of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 6.258 mg/100 mg of dry tissue after 7 days exposure and it was 5.008 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the RNA contents after exposure to the pesticides.

RNA content in whole soft body:

The RNA content in whole soft body of bivalves in control group at 7 days was 6.845 mg/100 mg of dry tissue for 21 days it was 6.811 mg/100 mg of dry tissue.

In the bivalves exposed to chronic concentration of indoxacarb (0.07811 ppm), at 7 days the RNA content in whole soft body was 5.679 mg/100 mg of dry tissue after 21 days exposure it was 4.820 mg/100 mg of dry tissue.

RNA content in digestive gland of bivalves exposed to chronic concentration of thiamethoxam (2.8422 ppm) was 5.884 mg/100 mg of dry tissue after 7 days exposure and it was 5.045 mg/100 mg of dry tissue after 21 days exposure. There was significant variation in the RNA contents after exposure to the pesticides.
Table: 3.1.1- Profiles of protein in different tissues of fresh water bivalve, *Parreysia cylindrica* after acute exposure to indoxacarb and thiamethoxam (Values are given in mg/100 mg of dry tissue).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>96hrs</td>
<td>24hrs</td>
<td>96hrs</td>
<td>24hrs</td>
<td>96hrs</td>
</tr>
<tr>
<td>Mantle</td>
<td>48.25 ± 0.12</td>
<td>47.69 ± 0.16</td>
<td>40.36 ± 0.31 (-16.3523) p&lt;0.05</td>
<td>38.56 ± 1.56 (-19.14) p&lt;0.01</td>
<td>43.36 ± 0.31 (-10.13) p&lt;0.05</td>
<td>36.26 ± 1.56 (-23.96) p&lt;0.01</td>
</tr>
<tr>
<td>Foot</td>
<td>68.91 ± 0.94</td>
<td>68.212 ± 1.26</td>
<td>57.91 ± 1.21 (-15.96) p&lt;0.05</td>
<td>50.91 ± 1.21 (-25.36) p&lt;0.01</td>
<td>60.26 ± 0.97 (-12.55) p&lt;0.05</td>
<td>53.01 ± 1.26 (-22.28) p&lt;0.01</td>
</tr>
<tr>
<td>Gill</td>
<td>54.11 ± 1.25</td>
<td>53.74 ± 0.92</td>
<td>43.86 ± 0.95 (-18.94) p&lt;0.01</td>
<td>38.26 ± 1.26 (-28.8053) p&lt;0.01</td>
<td>48.05 ± 1.16 (-11.19) p&lt;0.05</td>
<td>40.12 ± 0.96 (-23.48) p&lt;0.01</td>
</tr>
<tr>
<td>Digestive gland</td>
<td>51.31 ± 0.96</td>
<td>51.216 ± 1.26</td>
<td>38.12 ± 0.76 (-25.7064) p&lt;0.01</td>
<td>29.89 ± 1.87 (-41.6393) p&lt;0.01</td>
<td>42.89 ± 1.34 (-16.41) p&lt;0.05</td>
<td>35.25 ± 0.16 (-31.17) p&lt;0.01</td>
</tr>
<tr>
<td>Whole soft body</td>
<td>58.21 ± 0.96</td>
<td>58.196 ± 1.56</td>
<td>42.46 ± 0.76 (-27.4007) p&lt;0.01</td>
<td>38.612 ± 1.16 (-33.6517) p&lt;0.01</td>
<td>49.965 ± 0.79 (-14.1642) p&lt;0.05</td>
<td>45.316 ± 1.31 (-22.1321) p&lt;0.01</td>
</tr>
</tbody>
</table>

Each value is the mean of five observations ± S.D.
Table: 3.1.2- Profiles of protein in different tissues of fresh water bivalve, *Parreysia cylindrica* after chronic exposure to indoxacarb and thiamethoxam (Values are given in mg/100 mg of dry tissue).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th></th>
<th>Indoxacarb (0.07811 ppm)</th>
<th></th>
<th>Thiamethoxam (2.8422 ppm )</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
<td>21 days</td>
<td>7 days</td>
<td>21 days</td>
<td>7 days</td>
<td>21 days</td>
</tr>
<tr>
<td>Mantle</td>
<td>49.25 ± 0.12</td>
<td>48.69 ± 0.16</td>
<td>39.66 ± 0.31 (-19.41)</td>
<td>31.58 ± 0.125 (-35.14)</td>
<td>41.50 ± 1.22 (-15.73)</td>
<td>34.21 ± 0.76 (-29.73)</td>
</tr>
<tr>
<td>Foot</td>
<td>69.11 ± 0.91</td>
<td>68.612 ± 1.256</td>
<td>56.91 ± 1.26 (-17.65)</td>
<td>45.12 ± 1.79 (-34.23)</td>
<td>59.75 ± 0.97 (-13.54)</td>
<td>49.561 ± 0.75 (-27.76)</td>
</tr>
<tr>
<td>Gill</td>
<td>54.91 ± 1.25</td>
<td>54.34 ± 0.92</td>
<td>42.076 ± 0.925 (-23.38)</td>
<td>33.619 ± 1.216 (-38.14)</td>
<td>45.61 ± 1.316 (-16.93)</td>
<td>37.076 ± 1.721 (-31.78)</td>
</tr>
<tr>
<td>Digestive gland</td>
<td>51.51 ± 0.96</td>
<td>51.216 ± 1.26</td>
<td>37.06 ± 0.76 (-28.05)</td>
<td>28.65 ± 1.32 (-44.15)</td>
<td>41.46 ± 1.34 (-19.70)</td>
<td>33.26 ± 0.75 (-35.11)</td>
</tr>
<tr>
<td>Whole soft body</td>
<td>59.23 ± 0.96</td>
<td>57.196 ± 1.56</td>
<td>45.216 ± 0.79 (-23.67)</td>
<td>36.161 ± 0.56 (-36.77)</td>
<td>46.965 ± 0.79 (-20.71)</td>
<td>42.02 ± 1.21 (-26.52)</td>
</tr>
</tbody>
</table>

Each value is the mean of five observations ± S.D.
Table: 3.1.3- Profiles of Free Amino Acids in different tissues of fresh water bivalve, *Parreysia cylindrica* after acute exposure to indoxacarb and thiamethoxam (Values are given in mg/ gm of dry tissue).

| Tissue          | Control |  | Indoxacarb |  | Thiamethoxam |  |
|---------------- |---------| | (0.3905ppm) |  | (14.2114ppm) |  |
|                | 24 hrs  | 96 hrs | 24 hrs     | 96 hrs | 24 hrs        | 96 hrs |
| Mantle         | 62.101 ±0.145 | 61.878 ±0.245 | 73.082 ±0.145 (+17.6825) p<0.01 | 77.572 ±0.345 (+25.3628) p<0.001 | 68.3705 ±0.045 (+10.0957) p<0.001 | 75.572 ±0.215 (+22.1306) p<0.001 |
| Foot           | 67.517±0.015 | 67.146±0.058 | 77.8356 ±0.127 (+15.2843) p<0.001 | 84.5265 ±0.3023 (+25.1929) p<0.001 | 76.0805 ±0.0872 (+12.55) p<0.001 | 82.572 ±0.145 (+22.9768) p<0.001 |
| Gill           | 71.642±0.123 | 70.796 ±0.023 | 84.987 ±0.085 (+18.6273) p<0.001 | 92.0325 ±0.133 (+28.4617) p<0.001 | 80.3965 ±0.121 (+11.7201) p<0.001 | 87.121 ±0.120 (+23.0592) p<0.001 |
| Digestive gland | 126.750±0.153 | 124.345 ±0.126 | 158.98 ±0.165 (+25.428) p<0.001 | 178.9596 ±0.162 (+41.191) p<0.001 | 148.1426 ±0.141 (+16.8778) p<0.001 | 163.386 ±0.132 (+31.3973) p<0.001 |
| Whole soft body | 65.086 ±0.132 | 64.245 ±0.134 | 83.1254 ±0.174 (+27.7163) p<0.001 | 87.1472 ±0.122 (+33.8955) p<0.001 | 74.523 ±0.171 (+14.4993) p<0.001 | 78.5667 ±0.122 (+22.2928) p<0.001 |

Each value is the mean of five observations ± S.D.
Table: 3.1.4- Profiles of Free Amino Acids in different tissues of fresh water bivalve, *Parreysia cylindrica* after chronic exposure to indoxacarb and thiamethoxam (Values are given in mg/ gm of dry tissue).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Indoxacarb (0.07811 ppm)</th>
<th>Thiamethoxam (2.8422 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
<td>21 days</td>
<td>7 days</td>
</tr>
<tr>
<td>Mantle</td>
<td>61.5743 ±0.122</td>
<td>58.3547 ±0.132</td>
<td>73.5874 ±0.124 (+19.506)</td>
</tr>
<tr>
<td>Foot</td>
<td>65.0125 ±0.133</td>
<td>62.3214 ±0.0889</td>
<td>76.1689 ±0.165 (+17.1607)</td>
</tr>
<tr>
<td>Gill</td>
<td>69.0231 ±0.016</td>
<td>68.0124 ±0.0789</td>
<td>84.9795 ±0.142 (+23.1175)</td>
</tr>
<tr>
<td>Digestive gland</td>
<td>124.8752 ±0.145</td>
<td>123.5487 ±0.899</td>
<td>160.8147 ±0.147 (+28.7803)</td>
</tr>
<tr>
<td>Whole soft body</td>
<td>63.2147 ±0.125</td>
<td>62.0874 ±0.126</td>
<td>78.2345 ±0.0765 (+23.76)</td>
</tr>
</tbody>
</table>

Each value is the mean of five observations ± S.D.
Table: 3.1.5- Profiles of L-ascorbic acid in different tissues of fresh water bivalve, *Parreysia cylindrica* after acute exposure to indoxacarb and thiamethoxam (Values are given in mg/100 mg of dry tissue).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Indoxacarb (0.3905 ppm)</th>
<th>Thiomethoxam (14.2114 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>96hrs</td>
<td>24hrs</td>
</tr>
<tr>
<td>Mantle</td>
<td>0.789 ± 0.041</td>
<td>0.751 ± 0.052</td>
<td>0.665 ± 0.045 (-15.71)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Foot</td>
<td>0.875 ± 0.054</td>
<td>0.845 ± 0.0654</td>
<td>0.745 ± 0.0845 (-14.85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Gill</td>
<td>0.749 ± 0.03</td>
<td>0.723 ± 0.0548</td>
<td>0.609 ± 0.0245 (-18.69)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Digestive gland</td>
<td>0.889 ± 0.046</td>
<td>0.874 ± 0.0784</td>
<td>0.645 ± 0.0658 (-27.4465)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Whole soft body</td>
<td>0.817 ± 0.037</td>
<td>0.811 ± 0.0654</td>
<td>0.6922 ± 0.0521 (-15.2998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Each value is the mean of five observations ± S.D.
Table: 3.1.6- Profiles of L-ascorbic acid in different tissues of fresh water bivalve, Parreysia cylindrica after chronic exposure to indoxacarb and thiamethoxam (Values are given in mg/100mg of dry tissue).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Indoxacarb (0.07811ppm)</th>
<th>Thiamethoxam (2.8422 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
<td>21 days</td>
<td>7 days</td>
</tr>
<tr>
<td>Mantle</td>
<td>0.799 ± 0.042</td>
<td>0.756 ± 0.0598</td>
<td>0.640 ±0.0984</td>
</tr>
<tr>
<td></td>
<td>(-19.89) p&lt;0.05</td>
<td>(-37.86) p&lt;0.01</td>
<td>(-14.64) p&lt;0.05</td>
</tr>
<tr>
<td>Foot</td>
<td>0.885 ± 0.064</td>
<td>0.845 ± 0.0845</td>
<td>0.716 ±0.0451</td>
</tr>
<tr>
<td></td>
<td>(-19.09) p&lt;0.05</td>
<td>(-33.84) p&lt;0.01</td>
<td>(-12.99) p&lt;0.05</td>
</tr>
<tr>
<td>Gill</td>
<td>0.789 ± 0.053</td>
<td>0.769 ± 0.0549</td>
<td>0.601 ±0.0621</td>
</tr>
<tr>
<td></td>
<td>(-23.82) p&lt;0.01</td>
<td>(-39.14) p&lt;0.01</td>
<td>(-18.02) p&lt;0.05</td>
</tr>
<tr>
<td>Digestive gland</td>
<td>0.889 ± 0.0416</td>
<td>0.871 ± 0.0751</td>
<td>0.635 ±0.0631</td>
</tr>
<tr>
<td></td>
<td>(-29.05) p&lt;0.01</td>
<td>(-43.28) p&lt;0.01</td>
<td>(-17.43) p&lt;0.05</td>
</tr>
<tr>
<td>Whole soft body</td>
<td>0.832 ± 0.067</td>
<td>0.811 ± 0.0751</td>
<td>0.623 ±0.0654</td>
</tr>
<tr>
<td></td>
<td>(-25.12) p&lt;0.01</td>
<td>(-34.40) p&lt;0.01</td>
<td>(-17.54) p&lt;0.05</td>
</tr>
</tbody>
</table>

Each value is the mean of five observations ±S.D.
Table: 3.1.7- Profiles of DNA in different tissues of fresh water bivalve, *Parreysia cylindrica* after acute exposure to indoxacarb and thiamethoxam (Values are given in mg/100mg of dry tissue).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Indoxacarb (0.3905 ppm)</th>
<th>Thiamethoxam (14.2114 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>96hrs</td>
<td>24hrs</td>
</tr>
<tr>
<td>Mantle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.345±0.0456</td>
<td>1.340±0.098</td>
<td>1.200±0.065 (-10.78)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Foot</td>
<td>2.577±0.062</td>
<td>2.549±0.0586</td>
<td>2.269±0.024 (-11.95)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Gill</td>
<td>1.238±0.084</td>
<td>1.19±0.095</td>
<td>1.035±0.045 (-16.39)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Digestive gland</td>
<td>2.318±0.085</td>
<td>2.298±0.113</td>
<td>1.683±0.124 (-27.39)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.01</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Whole soft body</td>
<td>2.371±0.122</td>
<td>2.365±0.136</td>
<td>2.105±0.114 (-11.21)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Each value is the mean of five observations ± S.D.
Table: 3.1.8- Profiles of DNA in different tissues of fresh water bivalve, *Parreysia cylindrica* after chronic exposure to indoxacarb and thiamethoxam (Values are given in mg/100 mg of dry tissue).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Indoxacarb (0.07811ppm)</th>
<th>Thiamethoxam (2.8422 ppm )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
<td>21 days</td>
<td>7 days</td>
</tr>
<tr>
<td>Mantle</td>
<td>1.455 ±0.021</td>
<td>1.421 ±0.0546</td>
<td>1.197±0.0896 (-17.73) p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.250±0.0684 (-14.0893) p&lt;0.05</td>
</tr>
<tr>
<td>Foot</td>
<td>2.612 ±0.087</td>
<td>2.598 ±0.0546</td>
<td>2.209±0.0845 (-15.42) p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.245±0.124 (-14.05) p&lt;0.05</td>
</tr>
<tr>
<td>Gill</td>
<td>1.249 ±0.071</td>
<td>1.238 ±0.0458</td>
<td>0.937±0.0568 (-27.64) p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.025±0.0431 (-17.93) p&lt;0.05</td>
</tr>
<tr>
<td>Digestive gland</td>
<td>2.297 ±0.074</td>
<td>2.256 ±0.123</td>
<td>1.420±0.0987 (-38.18) p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.487±0.0124 (-35.26) p&lt;0.01</td>
</tr>
<tr>
<td>Whole soft body</td>
<td>2.412 ±0.124</td>
<td>2.398 ±0.134</td>
<td>2.004±0.131 (-16.91) p&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.034±0.109 (-15.67) p&lt;0.05</td>
</tr>
</tbody>
</table>

Each value is the mean of five observations ± S.D.
Table: 3.1.9- Profiles of RNA in different tissues of fresh water bivalve, *Parreysia cylindrica* after acute exposure to indoxacarb and thiamethoxam (Values are given in mg/100mg of dry tissue).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Indoxacarb</th>
<th>Thiomethoxam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hrs</td>
<td>96hrs</td>
<td>24hrs</td>
</tr>
<tr>
<td>Mantle</td>
<td>4.895 ±0.321</td>
<td>4.781 ±0.215</td>
<td>4.056 ±0.348</td>
</tr>
<tr>
<td></td>
<td>(-17.13) p&lt;0.05</td>
<td>(-25.85) p&lt;0.01</td>
<td>(-11.0929) p&lt;0.05</td>
</tr>
<tr>
<td>Foot</td>
<td>5.671 ±0.242</td>
<td>5.612 ±0.324</td>
<td>4.875 ±0.412</td>
</tr>
<tr>
<td></td>
<td>(-14.03) p&lt;0.05</td>
<td>(-24.16) p&lt;0.01</td>
<td>(-9.6631) p&lt;0.05</td>
</tr>
<tr>
<td>Gill</td>
<td>6.136 ±0.218</td>
<td>6.111 ±0.564</td>
<td>5.069 ±0.654</td>
</tr>
<tr>
<td></td>
<td>(-17.38) p&lt;0.05</td>
<td>(-31.79) p&lt;0.01</td>
<td>(-7.2033) p&lt;0.05</td>
</tr>
<tr>
<td>Digestive gland</td>
<td>8.642 ±0.824</td>
<td>8.581 ±0.791</td>
<td>6.15 ±0.654</td>
</tr>
<tr>
<td></td>
<td>(-28.83) p&lt;0.01</td>
<td>(-45.86) p&lt;0.01</td>
<td>(-24.26) p&lt;0.05</td>
</tr>
<tr>
<td>Whole soft body</td>
<td>6.878 ±0.220</td>
<td>6.871 ±0.324</td>
<td>5.843 ±0.453</td>
</tr>
<tr>
<td></td>
<td>(-15.0479) p&lt;0.05</td>
<td>(-32.77) p&lt;0.01</td>
<td>(-11.13) p&lt;0.05</td>
</tr>
</tbody>
</table>

Each value is the mean of five observations ± S.D.
### Table: 3.1.10- Profiles of RNA in different tissues of fresh water bivalve, *Parreysia cylindrica* after chronic exposure to indoxacarb and thiamethoxam (Values are given in mg/100 mg of dry tissue).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Indoxacarb (0.07811 ppm)</th>
<th>Thiomethaxam (2.8422 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 days</td>
<td>21 days</td>
<td>7 days</td>
</tr>
<tr>
<td><strong>Mantle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.879±0.361</td>
<td>4.759±0.321</td>
<td>3.884±0.451 (-20.39, p&lt;0.05)</td>
</tr>
<tr>
<td><strong>Foot</strong></td>
<td>5.665±0.213</td>
<td>5.423±0.348</td>
<td>4.697±0.321 (-17.08, p&lt;0.05)</td>
</tr>
<tr>
<td><strong>Gill</strong></td>
<td>6.113±0.234</td>
<td>6.011±0.641</td>
<td>4.825±0.491 (-21.06, p&lt;0.05)</td>
</tr>
<tr>
<td><strong>Digestive gland</strong></td>
<td>8.594±0.564</td>
<td>8.486±0.645</td>
<td>5.852±0.713 (-31.19, p&lt;0.01)</td>
</tr>
<tr>
<td><strong>Whole soft body</strong></td>
<td>6.845±0.224</td>
<td>6.811±0.361</td>
<td>5.679±0.513 (-17.03, p&lt;0.05)</td>
</tr>
</tbody>
</table>

Each value is the mean of five observations ± S.D.
Fig. 1. Profiles of protein in different tissues of fresh water bivalve, Parreysia cylindrica after acute exposure to indoxacarb and thiamethoxam (Values are given in mg/100 mg of dry tissue).

Protein content at 24 hours

Protein content at 96 hours

Tissue
Fig. 2. Profiles of protein in different tissues of fresh water bivalve, *Parreysia cylindrica* after chronic exposure to indoxacarb and thiamethoxam (Values are given in mg/100 mg of dry tissue).
Fig. 3. Profiles of Free Amino acids in different tissues of fresh water bivalve, *Parreysia cylindrica* after acute exposure to indoxacarb and thiamethoxam (Values are given in mg/gm of dry tissue).
Fig. 4. Profiles of Free Amino acids in different tissues of fresh water bivalve, *Parreysia cylindrica* after chronic exposure to indoxacarb and thiamethoxam (Values are given in mg/gm of dry tissue).
Fig.5. Profiles of L-ascorbic acid in different tissues of fresh water bivalve, *Parreysia cylindrica* after acute exposure to indoxacarb and thiamethoxam (Values are given in mg/100 mg of dry tissue).
Fig. 6. Profiles of L-ascorbic acid in different tissues of fresh water bivalve, *Parreysia cylindrica* after chronic exposure to indoxacarb and thiamethoxam (Values are given in mg/100mg of dry tissue).

![L-ascorbic acid content at 7 days](image1)

![L-ascorbic acid content at 21 days](image2)
Fig. 7. Profiles of DNA in different tissues of fresh water bivalve, *Parreysia cylindrica* after acute exposure to indoxacarb and thiamethoxam (Values are given in mg/100mg of dry tissue).
Fig. 8. Profiles of DNA in different tissues of fresh water bivalve, *Parreysia cylindrica* after chronic exposure to indoxacarb and thiamethoxam (Values are given in mg/100 mg of dry tissue).
Fig. 9. Profiles of RNA in different tissues of fresh water bivalve, *Parreysia cylindrica* after acute exposure to indoxacarb and thiamethoxam (Values are given in mg/100mg of dry tissue).
Fig. 10. Profiles of RNA in different tissues of fresh water bivalve, *Parreysia cylindrica* after chronic exposure to indoxacarb and thiamethoxam (Values are given in mg/100 mg of dry tissue).
DISCUSSION

The pesticides and related chemicals originating from human activity or agricultural farming are discharged directly or indirectly into the receiving waters. The presence of these chemicals in the environment has become a global issue. The human population is expected to reach 9.3 billion by 2050 (U.S. Bureau of the Census, 2005), resulting in a significant increase of domestic and industrial sewage production and a high demand for potable water. The indiscriminate discharge of agricultural runoff, sewage to the aquatic environment as well as the occasional failing of sewage treatment plants to eliminate some chemicals has raised concerns about the future water quality in rivers, lakes, estuaries and ocean. These ecosystems are very important for many species, because they serve as a nursery area and as source of food and nutrients for fish and birds (Allanson and Baird, 1999). The many non-target organisms like fishes, bivalves, prawns and crabs etc of the freshwater ecosystem are adversely affected. Pollution is recognized as one of the most significant factors causing major declines in populations of freshwater species in many parts of the world (Winfield, 1992; Lawton and May, 1995; Maitland, 1995; Mason, 1996a). Nearly 70% of the freshwater mussel (Bivalvia: Unionidae) species in the United States are considered as endangered, threatened, or of special concern Williams et.al. (1993).

Pesticides cause stress to the aquatic organisms (Newell, 1973; Madhyastha, 1996) and change the metabolic activities causing metabolic disarrangement in the living system. Pesticide induced changes in biochemical composition of organs and the physiological state of metabolic activity of an organism reflects in the utilization of their biochemical energy to counteract the toxic stress (Swami et. al., 1983). Pesticides due to
their potential toxicity produce biochemical changes in the tissues and organs of exposed animals (Shastry and Sharma, 1979). During exposure, organism goes through a shift in all the metabolic processes to counteract the toxic effects by undergoing all protective measures. Toxic stress of any kind leads to changes in biochemical and physiological mechanism in the body of organism. In order to investigate the physiological changes after pesticide treatment, the study of changes in the biochemical constituents is the most fundamental tool. Biochemical studies are good parameters which help to see the effect of pesticide on biochemical composition of vital tissue of bivalve.

In terms of energy conservation, the organism would be expected to make compensatory adjustments for both gain and loss of energy in such a way that gain is maximized and loss is minimized in case of change in environmental condition (Bayne, 1973). Observations recorded in this study revealed that the body parts of *Parreysia cylindrica* might have got capacity to gear the metabolic substance during the variable stress depending upon type of effect of the stress factor, as was observed by Mane and Muley (1989) in *Lamellidens marginalis*.

Biochemical changes which are noticed, may be due to the response to pesticidal intoxication representing adaptive or regulatory mechanism of may be due to pathological effect. The organism undergoes a shift in all the metabolic processes and defends itself from the toxic effects by undergoing all protective measures. The pesticide gives rise to alterations in the metabolic and physiological activity both after short and long term exposure.
Changes in protein content:

Protein is an important organic constituent that plays an important role in the metabolism. It is important as an integral part of cell membrane, and intracellular and extracellular soluble proteins and enzymes. Interactions occurring during protein metabolism in protein, amino acids, enzymes and co-enzymes were studied by Harper et al., (1978).

Proteins play a key role in cellular metabolism hence, protein synthesis or hydrolysis is considered as a premier biochemical indicator of stress. It is influenced by large number of exogenous factors either through a decrease in protein synthesizing capacity of endoplasmic reticulum or increase in their turnover in the cells (Syverson, 1977; 1981).

In the present study exhibits decrease in over all protein content in mantle, foot, gills, digestive glands and whole body tissues of Parreysia cylindrica when exposed to acute and chronic dose of pesticide, indoxacarb and thiamethoxam. The depletion was maximum in digestive glands than in mantle, foot gills and whole body. The higher depletion of protein in the digestive gland might be due to high metabolic potency and efficiency of the gland when compared to other tissues like mantle, foot gills and whole body of the bivalve. The digestive gland is site of action of pollutant in the body of bivalve or digestive gland seems to be the main site of degradation and detoxification of pesticides and hence has the largest demand of energy for the metabolic processes resulting into increasing utilization of protein to meet energy demand. The higher degradation of protein in digestive gland provided better indication of the extent of

The decrease in amount of protein content in all tissues after exposure to indoxacarb and thiamethoxam is attributed to the impairment of protein synthesis and increase in the rate of their degradation to amino acid which may be fed to TCA cycle through aminotransferase probable to crop with the high energy demands to cope with the stress conditions (Kabeer et al., 1978). Jha (1988) and Waykar and Lomte (2001) supported the idea of consumption of amino acid for metabolic processes as energy source. According to SivaprasadRao and Ramanarao (1980) depletion of protein in pollutant treated animals might be due to enhanced proteolytic enzymes activity. Increase in protease enzyme activity also supported depletion of protein content (Srinivas and Purushotam Rao, 1987). They observed increased protease activity in Bombayx mori. Waykar and Lomte (2002, 2004) observed increased protease activity in mollusc after exposure to pesticide. According to Abel (1974) the depletion of protein may be due to alterations of membrane permeability. Another probability was that pollutant might block protein synthesis (Passow et al., 1961).

Soivio and Oikari (1976) reported that pesticide stress causes electrolyte imbalance and place one of the largest demands for energy on the metabolic processes of the animal. A marked fall in the protein level in all the tissues indicates a rapid initiation of breakdown of protein. To meet energy demands during toxic stress, mobilization of protein might have taken place (Lomte and Alam, 1982). It is possible that in tissues exposed to pesticide a high demand of energy for maintenance of osmotic balance which
resulted in decreased level protein was observed by Muley and Mane (1987) in *Lamellidens marginalis*.

Pesticide induced changes in biochemical composition of organs and the physiological state of metabolic activity of an organism reflects in the utilization of their biochemical energy to counteract the toxic stress (Swami *et. al.*, 1983). Pesticide toxicity reflects a suppressing effect on various biochemical constituents such as proteins, nucleic acids, lipids etc in the molluscs. Proteins can be expected to be involved in the compensatory mechanism of stressed organisms (Ramalingam and Ramalingam, 1982).

Any undesirable change in the surrounding of organism influences the turnover and ultimately several complex biological functions. A marked depletion in protein content of aquatic invertebrates on exposure to toxic substances was reported by many investigators. Ramana Rao and Ramamurthi (1978) reported the depletion in protein content in the tissues of *Pila globosa* after exposure to pesticides. Lomte and Alam (1982) observed the decline in protein level in *Bellamiya bengalensis* after pesticide stress. Lomte and Alam (1982) studied effect of Malathion on the biochemical components of the prosobranch, *Belamia bengalensis* and reported that the decrease in glycogen, protein and lipid under pesticide stress. Shivprasad (1981) studied the effect of methyl parathion pesticide on *Pila globosa* and reported that the depletion in protein level in different tissues due to enhanced proteolytic enzyme activity and decreased protein synthesis.

The depletion of tissue protein was due to diversification of energy to meet the impending energy demand under toxic stress (Vincent *et. al.*, 1995).
Muley and Mane (1995) observed that, the freshwater bivalve *L. marginalis* when exposed to sub lethal dose of endosulfan, showed depletion of protein content of almost all tissues. The decrease in protein content may be due to altered size of pores in membrane (Abel, 1974) or diminished protein synthesis (Reddy, 1979). Nagpure and Zambare (2005) observed that on acute and chronic exposure to tetracycline and chloramphenicol, *L. corrianus* showed decrease in protein levels, in proportion with the period of exposure. The decrease in average total protein content of tissue after treatment suggests enhancement of proteolysis to meet the high energy demands under heavy metal or other stress. Singh and Agarwal (1996) reported significant decrease in endogenous levels of protein in foot tissue in *Lymnea accuminata* on exposure to deltamethrin.

The percent decrease of proteins after acute and chronic exposure of pesticides might be due to over exertion or activity of muscle under pesticide stress. Similar results were reported by several workers Baig Azahar *et. al.*, (1991), Cope *et. al.*, (1970), Kabeer *et. al.*, (1983) and Sivaprasada Rao (1980), when studied the toxicity of different pesticides. Tiwari *et. al.*, (2004) worked on toxic and sub lethal effect of oleandrin on biochemical parameters of freshwater fish, air breathing murrels *Channa punctatus* and observed that the significant alteration in the level of total protein, free amino acid, nucleic acid, glycogen, lactate, enzyme protease, phosphatase, alanine aminotransferase, aspirate aminotransferase and acetyl cholinesterase activity in liver and muscle tissues. Durga and Veeraiah (2002) worked on effect of cypermethrin on protein metabolism of the fish *Labeo rohita* observed that the total protein level decreased in all the tissues tested where as the free amino acid levels were increased.
Biochemical changes induced by pesticide stress lead to metabolic disturbances, inhibition of important enzymes, retardation of growth and reduction in the fecundity and longevity of the organism.

Parate and Kulkarni (2003) were of the opinion that, depletion of the protein may be due to its utilization for the production of energy to alleviate the pesticide stress and to prevent fatigue which may occur due to the effect of pesticides. Waykar (1999) also observed increased protease activity in fresh water bivalve, Parreysia cylindrica after pesticide treatment. The proteolytic activity seems to be high due to increased transminase activity (Subbarao, 1983) by which amino acids can be catalyzed in the T.C.A. cycle as Keto acids (Kabeer et.al., 1978). A marked fall in the protein level in all the tissues indicates a rapid initiation of breakdown of protein. To meet the energy demand during toxic stress, mobilization of protein might have taken place (Lomte and Alam, 1982). Muley and Lomte (1992, 1993 and 1995) reported decreased protein content in different tissues of snail, Thiara tuberculata, after pesticidal treatment.

exposure to chromium in three different tissue viz. adductor muscles, gills and mantle of fresh water mussel, *Lamellidens marginalis*. The decrease was more pronounced in the gills followed by adductor muscles and mantle. Total, structural and soluble proteins decreased significantly and to continence, this the levels of amino acids and protease activity increased in all the tissues of snail at all time points examined. Kulkarni *et al.*, (2005) found significant decrease in total protein content in foot, hepatopancreas and gills of the fresh water mussel, *Lamellidens corrianus* on exposure to organochlorine insecticide, hildan. They further concluded that, decline in protein content indicates intensive proteolysis which is followed by corresponding decrease in total free amino acids. The decrease in the tissue lipid and proteins might be partly due to their utilization in cell repair and tissue organization with the formation of lipoproteins which are important cellular constituents of cell membranes and cell organelles present in cytoplasm (Harper, 1963). Kamble *et al.*, (2010) reported the significant decrease in the protein, glycogen, lactic acid and cholesterol contents, in gills, hepatopancreas, gonads, muscle, mantle and foot. A significant decrease was reported in the protein content in almost all tissues in *Channa punctatus* when exposed to sub lethal and lethal concentration of fenvalerate (Tilak *et al.*, 2003). According to Lynch *et al.*, (1969) decreased level of protein could be due to the reduction in protein synthesis because of liver cirrhosis.

Abdul Naveed *et al.*, (2006) reported that, serum biochemical parameters of fish *Channa punctatus* could be altered on treatment with sub lethal concentration of triazophos and found decreased level of protein. They concluded that the level of the protein decreased due to increased proteolytic activity which might be the cause of
increased amino acid pool during the pesticide exposure period. Swami et. al., (1983) noted decrease in total protein content in foot, mantle and hepatopancreas of the fresh water mussel, Lamellidens marginalis due to metacid and flodit exposure. Kulkarni et. al., (2005) found significant decrease in total protein content in foot, hepatopancreas and gills of the fresh water mussel, Lamellidens corrianus on exposure to the sub lethal concentration of organochlorine insecticide, hildan. They further concluded that, decline in protein content indicates intensive proteolysis which is followed by corresponding decrease in total free amino acids.

**Changes in amino acid content:**

In the present study a marked increase in the level of the free amino acid in the mantle, foot, gill, digestive gland and whole body tissue of fresh water bivalve Parreysia cylindrica was found after acute and chronic exposure to pesticides.

This increase in the free amino acid content may partly be due to diminished utilization of amino acid in the tissues or due to their enhanced synthesis from other sources. The high concentration of free amino acid is believed to play an important role in osmoregulation (Bishope et al, 1962). Free amino acid is known to maintain the osmotic balance and they serve as osmoregulators in mollusk (Florkin and Schoffeniels, 1964).

Free amino acid is concerned with energy production (Wyatt, 1972). Bursell (1953) reported that certain amino acids might play an important role as energy source. Tissue protein depletion vis-à-vis an increase in free amino acid levels and in the activity of transaminases represented gluconeogenesis from protein source during poisoning in prawn (Ramanarao et al., 1990). The loss of tissue protein, hyperproteinemia and an
elevation of free amino acid pool was observed during poisoning. Sarvesh kumar et al. (1992) suggested that it may have similar function in fresh water bivalve, too to derive extra energy to meet the stressful situation during their toxic exposure.

Schafer (1966), Goldstein et al. (1975), Ahokas and Sorg (1977), Juerss (1980) and Okuma and Abe (1994) studied effects of pollutant on free amino acid content in fish and also reported that during pollution stress, free amino acid content increases as osmorgulatory homeostasis. Bhagyalakshmi (1981) observed increases in amino acid level after pesticide treatment in crab. Sivaprasad Rao et al. (1998) reported increased free amino acid level in Pila globosa after pesticide treatment. Rajeswara Rao et al. (1982) reported increased free amino acid level after exposure to pesticide phenthoate in Pila globosa. Dikshit (1975) have also observed changes in free amino acid level after exposure to pesticides.

Changes in ascorbic acid content:

Ascorbic acid is a sugar acid with antioxidant properties. One form of ascorbic acid is commonly known as vitamin C. The name is derived from α- (meaning "no") and scorbutus (scurvy), the disease caused by a deficiency of vitamin C. In 1937 the Nobel Prize for chemistry was awarded to Walter Haworth for his work in determining the structure of ascorbic acid (shared with Paul Karrer who received his award for work on vitamin), and the prize for Physiology or Medicine that year went to Albert Szent-Gyorgyi for his studies of the biological functions of L-ascorbic acid. At the time of its discovery in the 1920s, it was called hexuronic acid by some researchers Svirstbel, Joseph Louis; Szent-Gyorgyi, Albert (1932).
Ascorbic acid (L,3-ketothreohexuronic acid lactone), commonly known as vitamin C, performs antioxidative functions in vivo by serving as a hydrogen ion donor at various metabolic sites. It plays an important role in the metabolism and the detoxification of many endogenous and exogenous compounds. The ascorbic acid has potential role to reduce the activity of free radical-induced reactions (Holloway, 1984). L-Ascorbic acid is a powerful antioxidant which plays an important role in intracellular oxidation-reduction system and in binding of free radicals produced endogenously (Laurence et al., 1977). L-Ascorbic acid reduced the clastogenic effects generated by certain chemical agents in the vivo and vitro assays (Amare-Mokrane et al., 1996, Khan et al., 1996). According to Edgar (1974), L-Ascorbic acid possesses substantial nucleophilic property and ascorbate might protect against electrophilic attack on cellular DNA by intercepting reactive agents or ascorbic anion radical with a high extent of unpaired electron delocalization which is the responsible for the scavenging of free radicals (Bieski, 1982).

L-Ascorbic acid has much significance in the body of animals. Ascorbic acid appears to be multi-functional; its role in fresh water bivalve received little attention (Chinoy and Seethalakshmi, 1977; Kachole et al, 1977; Ali et al., 1983).

The ascorbic acid content decreased in mantle, foot, gill, digestive gland and whole body due to pesticide stress. Somasundaram et al. (1978) reported that concentration of ascorbic acid depends upon the physiological state of organism.

Acute poisoning by pesticides certainly represents stress (Motton and La Ham, 1969). McCann and Jasper (1972) reported haemorrhage and vertebral injuries in fishes exposed to high level of various pesticides. Stress caused alteration in the normal physiology of the animal leading to enhanced utilization and mobilization of ascorbic acid (Chinoy and Kamalakumari, 1976) as ascorbic acid is recognized as antistress factor (Kutsky 1973).

The decreased ascorbic acid content might be due to impairment in its synthesis. This suggest that in Parreysia cylindrica, the ascorbic acid might have induced hepatic mixed function of oxidase system and played important role of biotransformation of toxic substances in to non toxic as observed by Kachole et al (1977), Ali and Ilyas (1981). The diminution might be due to decreased biosynthesis and increased catabolism as was observed by (Banerjee and Basu 1975). This also suggested that increased demand of energy being provided by utilization of ascorbic acid, due to pesticide stress, as observed by (Gorbunova, 1966).

Ali et al (1983) and Gould (1963) reported the accumulation of ascorbic acid at the site of wound healing. Jadhav et al. (1996) observed 10-66 % decrease in ascorbic acid content on acute exposure and 45 - 73% decrease on chronic exposure of fresh water bivalve, Corbicula striatella to carbaryl. Shanta and Motellica (1962) stated that, concentration of ascorbic acid is dependent on the physiological state of organism. Chinoy and Kamalakumari (1976) stated that, stress causes alterations in the normal.
physiology of animal leading to enhanced utilization and mobilization of ascorbic acid as ascorbic acid is recognized as antistress factor (Kutsky, 1973).

Dedemeyer (1969) observed a decrease in the ascorbic acid in the kidney of *Salmonids* on exposure to stressful situation. The domination might be due to decreased biosynthesis and increased catabolism as was observed by Bannerjee and Basu (1975). Roychudhari and Mukherjee (1976) recorded decrease in ascorbic acid content in testes at low temperature in *Rattus rattus* and *Bufo bufo* while Wilson and Poe (1973) demonstrated its anti-scorbutic role in fish, *Clarius batracus*,

Padmaja and Reddy (1998) found decrease in ascorbic acid content in heavy metal exposed *Anabas testudinus*, probably for detoxification and restoration to normal level during recovery. During chromium-induced toxicity, ascorbic acid may maintain osmo-regulation by altering ATPase activity in the gills for the synthesis of metallothiones (Padmaja and Reddy, 1998).

Waykar *et al* (2001) found decreases in ascorbic acid content in whole body, mantle, gills, digestive glands and increase in foot after acute and chronic treatment of cypermethrin to *Parreysia cylindrica*. These observations suggest that, ascorbic acid shifted to the foot due to increased demand of energy and fatigue retardant. Decrease in ascorbic acid content might be due to the impairment in its synthesis on pesticide stress.

As per present work is concerned, decrease in ascorbic acid content in different tissues of *Parreysia cylindrica* might be due to its involvement in detoxification and repairing of injuries in tissues which occurred due to pesticide stress.

The ascorbic acid content was decreased after acute exposure to mercuric chloride and sodium arsenate in freshwater bivalve, *Lamellidens corrianus* (Gulbhile, 2006).
Ascorbic acid is known to provide an antimutagenic property against a wide variety of chemicals (Sweetman et al., 1997). Due to its nucleophilic properties, it binds with mercury ions and reduces Hg induced DNA damage (Rao et al., 2001). In addition, it performs various important physiological functions such as collagen synthesis and formation of connective tissues (Wilson and Poe, 1973).

**Changes in the DNA content:**

It was observed that after chronic exposure of endosulfan, there was significant decrease in the DNA content in gill, gonad and digestive gland of experimental bivalves as compared to those of control bivalves.

DNA content, the index of capacity of an organism for protein synthesis in the different stress conditions was affected by heavy metals or any toxic metals or pesticides. Patiashvili et al. (1989) reported that copper ions introduced into asides tumors penetrate the nucleic acid (DNA) and damage it, causing incardinating of the chromatin structure, copper associates with DNA at higher copper concentrations. (Marion et al., 1987) and simultaneous changes in Ca, Mg, Fe, Cu and Zn concentrations in cultured human lymphocytes affected thymidine incorporation and surface antigens of human T and B lymphocytes (Carpentieri, 1987).

Black et al (1996) observed significant DNA strand breakage in the foot tissue from *Anodonta grandis* exposed to lead. They suggested that a threshold effect for DNA damage and repair resulting from Pb exposure were by repair of DNA strand breaks that may occur only if certain body burden or exposure duration has been achieved.
Tong Lu et al (2001) observed that approximately 60 genes (10%) were differentially expressed in arsenic exposed human livers compared to controls. The differentially expressed genes induced those involved in cell cycle regulation, apoptosis, DNA damage response, and intermediate filaments. The observed gene alterations appear to be reflective of hepatic degenerative lesions seen in the arsenic exposed patients. This array analysis revealed important patterns of aberrant gene expression occurring with arsenic exposure in human liver. Aberrant expressions of several genes were consistent with the results of array analysis of chronic arsenic exposed mouse livers and chronic arsenic - transformed rat liver cells.

They suggested that clearly a variety of gene expression changes might play an integral role in arsenic hepatotoxicity and possibly carcinogenesis. The metals may be carcinogenic because of their ability to generate reactive oxygen species and other reactive intermediates or react directly with DNA (Brien et al., 2003). In deed, several transition metals can generate reactive oxygen species in biologic fluids at physiologic pH, Moreover Cr (VI) is a better carcinogen than Cr (III) and yet Cr (III) is much better in DNA binding reactions than nickel or Cr (VI), Brien et al (2003). In addition the high radioactive metals iron and copper, which also bind to DNA more avidly than does Ni (II) and are only weakly carcinogenic, if at all (Desoize, 2003; Theophanides and Anastassopoulou 2002).

Another explanation may be based on the known ability of carcinogenic metals to facilitate DNA damage through inhibition of DNA repair enzymes (Hartwing et al, 2002) or binding to histones. In both cases, it is assumed that the ultimate target of free radicals or metals is DNA and that the mechanisms of carcinogenesis must include genotoxic
effects. However, metal such as Ni (II) is only weakly genotoxic and mutagenic (Mayer et al., 1998).

Rao et al. (1998) studied the effect of Fluoride toxicity on the nucleic acid contents of freshwater crab, Barytelphusa cunicularis. They observed that the level of DNA in muscles and hepatopancreas were found to be elevated initially and then a gradual decrease was noted in gills, testes and ovaries.

The decreased levels of DNA and RNA were observed by various investigators, Asifa Parveen and Vasantha (1986) in Clarius batrachus, Patil and Lomte (1989) in Mythima seperata, Choudhari et al. (1993) in Thiara lineata under various different toxic stresses. The cellular degradation rapid histolysis and decreased rate of protein synthesis are possible reasons behind this. As compared and supported by above literature, the present investigation of the acute exposure of mercury and arsenic to bivalves L. corrianus, showed decreased DNA contents compared with control bivalves, and those exposed to heavy metals with caffeine. The bivalves showed faster recovery due to caffeine, as compared with those recovered in natural water.

**Changes in the RNA contents:**

RNA polymerase binds the binding site especially to its DNA template, binds its nucleotide and primer substrates, forms a new phosphodiester bond and elongates the growing RNA.

The present investigation shows the interaction of pesticide and RNA. It shows that the pesticides reduced. The decrease in RNA on exposure to endosulfan may be due to damage in DNA, poor rate of synthesis of enzymes necessary for transcription or increased catabolism of RNA due to their abnormalities on interactions to pesticide.

Generally more energy is needed to mitigate any stress conditions. This may be obtained from protein, ascorbic acid. Hence in the present investigation, the reduction of protein, ascorbic acid, DNA and RNA concentrations can be taken as meaningful biochemical indices of pesticide toxicity to assess the extent of pollution of the aquatic environment.
SUMMARY

• In the present investigation impact of pesticides indoxacarb and thiamethoxam on protein, free amino acid, ascorbic acid, DNA and, RNA contents in the freshwater bivalve, Parreysia cylindrica was studied.

• Protein, free amino acid, L-ascorbic acid, DNA and RNA contents in mantle, gills, foot, digestive gland and whole soft body tissue of freshwater bivalve, Parreysia cylindrica were estimated after acute and chronic exposures to sublethal concentration of indoxacarb and thiamethoxam.

• There was significant decrease in over all protein content in mantle, foot gills, digestive glands and whole body tissue of Parreysia cylindrica due to acute and chronic exposure to pesticides indoxacarb and thiamethoxam. The depletion was maximum in digestive glands than in mantle, gills, foot and whole body tissue. Pesticidal stress might have increased the proteolysis activities in the cells.

• Depletion was also observed in ascorbic acid levels in mantle, foot, gills, digestive glands and whole body tissues of fresh water bivalve, Parreysia cylindrica on acute and chronic treatment of pesticides indoxacarb and thiamethoxam. It might be due to involvement of ascorbic acid in detoxifying mechanism.

• Depletion was also observed in DNA levels in mantle, foot, gills, digestive glands and whole body tissues of fresh water bivalve, Parreysia cylindrica on acute and chronic treatment of pesticides indoxacarb and thiamethoxam. It might be due to depletion of some nuclear DNA or damage of mitochondria and their DNA.
Depletion in RNA levels in mantle, foot, gills, digestive glands and whole body tissues of fresh water bivalve, *Parreysia cylindrica* on acute and chronic treatment of pesticides indoxacarb and thiamethoxam indicates their effect on the RNA transcription, or damage of rRNA or tRNA in the cytoplasm.
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