CHAPTER - 1

General Introduction
The art of silk production is "Sericulture", an important cottage industry based on agroforestry earning foreign exchange worth about Rs.1500 crores per annum. Presently Sericulture is practiced in more than 60,000 villages providing gainful employment, economic development and improvement in the quality of life to the people approximately 58 lakhs, most of them being small and marginal farmers. India has the distinction of cultivating all the four commercially known varieties of silk, namely, Mulberry, Tasar, Eri and Muga. The world raw silk production (Mulberry and non mulberry) about 125629 MT is mainly from two countries, China and India. China leads the world with silk production of 102560 MT or 81.6% of the produce. India ranks second in respect of world raw silk production. It is this position, as one of only two major silk producers in the world, and from its employment potential, that sericulture and silk derive their importance in the Indian textile map. Policy decisions are defined mainly by these two considerations.

It is well known that the domestic production of raw silk is not adequate to meet the domestic and export demand. It is estimated
that against the demand of around 26,000 tonnes per annum the domestic production is around 16500 tonnes. The gap of nearly 9500 tonnes in demand is mainly on account of the fact that high grade mulberry raw silk is not being produced in the country to the extent required by the industry. This quality of mulberry raw silk is basically required in the power loom industry, for export purposes, and to some extent in the hand loom industry for warp purposes. The present global production is fluctuating around 70,000 to 90,000 MT and the demand for silk is annually increasing by 5%. With the increase in population and also with the increased demand for fashionable clothing items due to fast changing fashion designs in developed countries, the demand for silk is bound to increase even more.

For increasing the silk production, we require highly productive mulberry varieties and silkworm races and also races tolerant to adverse climatic conditions and diseases, that can come mainly from the Seri cultural germplasm resources and also from the wild relatives of *Bombyx* available in the natural habitats. However the success of a silkworm crop depends on the quality and the quantity of the mulberry leaves. Since sericulture is practiced in diverse agro climatic zones of the country, there is a possibility of variations in the nutrients and the trace elements available in the mulberry leaves depending on the different soil constituents. The elements such as Fe, Mg, Na, K, S, Se and F can be acquired by the plants, is readily magnified in the food chain, and is known to influence the physiology
and biochemical metabolism in wild life, domestic animals and man. For instance, higher than the usual amounts of certain trace elements are implicated as toxic agents. At the other extreme, low levels of essential elements cause deficiency diseases in flora and fauna. Therefore it is imperative to study the possible role of different essential trace elements in plant and animals. Selenium is an essential trace nutrient important to humans and most other animals as an antioxidant, functioning as the metal co-factor for important enzymatic activity requiring glutathione peroxidase (Mayland, 1994). Selenium was isolated by Berzilius in 1818. Much work is needed to clarify the chemistry of selenium in regard to dietary forms, their metabolic fate, and the forms in which selenium functions as an essential element. Comprehensive and systematic review of the various aspects of organic chemistry of selenium, was made by Campbell et al., (1952). The nomenclature for organo- selenium compounds is similar to that of sulphur compounds. Selenium compounds exert their biological effects either directly or by being incorporated into enzymes and other bioactive proteins. The main inorganic dietary form of selenium is sodium selenite. In the organic forms selenomethionine and selenocysteine, where sulphur is replaced by selenium atom in the aminoacids methionine and cysteine.
Considerable evidence has been presented to indicate that dimethylselenite is a metabolic product of inorganic selenium in biological system (Challenger, 1951).

\[ \text{Na}_2\text{Se} + 2\text{CH}_3\text{OSO}_3\text{Na} \rightarrow (\text{Se(CH}_3)_2 + 2\text{Na}_2\text{SO}_4 \]

Selenium analogs of antifungal bacteriostatic and carcinostatic thiocompounds were synthesized and the biological effectiveness, was studied by a few investigators (Mautner et al., 1956; Sawicki and Carr, 1957; Carr et al., 1958; Dingwall, 1962)

Selenium is found in man's natural environment (Fig.1) and under normal conditions, is present in the atmosphere, soils, igneous rocks, sedimentary rocks, volcanic gases, oceans, ground and running
waters, in our food and body. It enters in the food chain by several routes but mainly through water. Much selenium in our environment comes from modern industry and commerce and the natural selenium chain is supplemented in selenium by man made chain. Phytoremediation programs have been made for selenium contaminated soils, including a selection of agricultural and weed species (Nyberg, 1991; Parker and Paise, 1994; Wu et al., 1996; Banuelos et al., 1997).
Plant foods can be regarded as the major dietary sources of selenium in most countries throughout the world. Food is the main source of selenium for the mammalian organism. The selenium is concentrated variably in varied sources but the concentrations may not exceed 100ppm in any material. The igneous rocks contain 0.09 ppm selenium. The volatile nature of selenium is a means of dispersal of the element. So that large areas of soil surfaces may be provided with small quantities of the element, when the volatile selenides come to the earth in rain and snowfall. The sedimentary rocks cover more than 3/4th of the land surface of the earth also has the selenium to the extent of 0.1 – 1ppm fossil fuels, dust coming from the air conditioning filters varies in its selenium concentrations and amounts to 0.05ppm. Pyritiferous shales have 1 – 10 ppm of selenium. Lateritic soils of United States contain 0.5 – 2.4ppm. Fossil, fuel and coal are the principle source of selenium in the atmosphere. Selenium enters the soil primarily as a result of the weathering of selenium containing rocks, volcanic activity and dusts. The combustion of coal and oil results in the release of selenium as SeO₂ and this form is generally reduced to elemental selenium. Eventhough, selenium is toxic to the organisms in higher concentration, it is predominantly used for production of photocells, rectifiers and semiconductors and in glass rubber and chemical industries. It is commonly produced as a byproduct of copper refining, roasting copper ore "slimes" with soda ash or sulphuric acid. Burning coal can produce selenium compounds and oil also releases selenium into the environment and small
particles in the air settle to the ground or are taken out of the air in rain. Selenium compounds deposited in agriculture fields from fertilizer use can be carried in irrigation drainage water easily take up selenium compounds from water and concentrate them. It also can build up in animals that eat plants or other animals with high levels of selenium.

The important difference in chemistry of selenium and sulphur is that quadrivalent selenium in selenite tends to undergo reduction, but quadrivalent sulphur in sulphite tends to undergo oxidation. This difference in their chemistry results in having different roles in biological system (Levander, 1997). In 1957, it was discovered as an essential trace element for animals and human. Although the toxic effects of selenium have been recognized much earlier than the nutritional properties, the exact mechanism of the manifestation of selenium toxicity in animals is not yet understood. However research on the nutritional aspects of selenium and its interaction with other nutrients and/or environmental chemicals has been comprehensively reviewed. Selenium is a semiconductor with photoconductivity i.e., excitation with electromagnetic radiation can markedly increase by its conductivity. This property was made selenium compound to become useful in the production of photocells and xerography. Elemental selenium can exist as selenium dioxide (SeO₂), selenious acid (H₂SeO₃) or as selenite (SeO₃²⁻).
Seleniferous soils were classified into two types with respect to the availability of selenium to plants. (1) Those that are alkaline and fairly any can support plant selenium concentration great enough to be toxic to animals, (2) Those acidic and moist are found to be non-toxic to plants. Widespread seleniferous vegetation has been found from Mexico through the United States into Canada (Fig. 2).

Fig. 2 Map of the United States showing distribution of seleniferous vegetation in regions considered to be seleniferous and distribution of white muscle disease of sheep in non-seleniferous regions (Muth and Allaway, 1963)

In other parts of the world seleniferous vegetation has also found in the countries like Australia, Ireland, Israel, South Africa and Venezuela. The accumulation of selenium is depends on specious and
soil type. It is known that a South American tree *Leyceylhis ollaria* bears a nut reported to contain as much as 18,000 ppm. of selenium; in Venezuela, ingestion of these nuts has caused severe toxicity symptoms and death in human beings. However, extremely small amounts of selenium ranging from 0.33, 1, 3 and 9 ppm of selenium stimulated the growth in *Astragalus racemosus* (Trelease and Beath, 1949)(Fig.3).

![Selenium growth stimulation in Astragalus racemosus](image)

*Fig. 3: Selenium growth stimulation in Astragalus racemosus* 

Most reviews on selenium biochemistry emphasize primarily on the aspects of selenium deficiency, rather than selenium toxicity (Shamberger, 1985). In recent years the toxic effects are associated with nutritional overexposure to selenium in humans and animals have been described and selenium is found to be an essential micronutrient that has major role to play in health and disease. The
pathology in acute toxicity is widespread necrosis, hemorrhage and death which are primarily due to hypoxia and secondarily due to lesions in the lungs. The most serious effect of selenium intoxication in adults is on the nervous system. It acts as an irritant to eye, skin, and respiratory tract and also causes gastrointestinal disturbances (Frost and Hish, 1975). The presence of selenium in higher concentrations than normal may alter the metabolic functions. The dietary requirement of selenium to man was estimated to be 0.4 – 0.19 mg / kg food. The body develops defensive mechanism such as antioxidants to control levels of free radicals as they can damage cells and contribute to the development of some chronic diseases (Combs et al., 1997). It is equally important for normal functioning of the immune system and thyroid gland or the human body (Levander, 1997; Arthur, 1991; Corvillain et al. 1993).

Metabolic disorders due to selenium inadequacy have been recognized practically in all the major livestock producing countries of the world. Some parts in China are associated with the selenium deficiency, where the selenium in the soil is low. In animals dietary selenium deficiency induces a number of pathological changes such as diminished growth and increased mortality in quail, pancreatic fibrosis and exudative diathesis in chicks, hepatic necrosis in rats and white muscle disease in cattle and sheep (Martin and Gerlackh, 1969). Selenium is responsible for the cause of Keshan diseases under its deficiency (Levander and Beck, 1997). This condition in men arises
when their dietary intake is less than 19 mcg and 13 mcg for women (Levander, 1991). Institute of Medicine Food and Nutrition Board, Washington (1996), revealed the recommended dietary allowances (RDA) for all ages as shown in Table 1.

**Table 1: Recommended dietary allowance for selenium for adults**

<table>
<thead>
<tr>
<th>Life Stages</th>
<th>Men</th>
<th>Women</th>
<th>Pregnancy</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 19 Years</td>
<td>55 mcg</td>
<td>55 mcg</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>All Ages</td>
<td>---</td>
<td>---</td>
<td>60 mcg</td>
<td>70 mcg</td>
</tr>
</tbody>
</table>

**Source:** Institute of Medicine, Food and Nutrition Board, Washington (1996).

Man is exposed to selenium through food, drinking water and by living near a selenium rich area. Higher doses of selenium could be fatal leading to chronic selenosis. The levels of selenium in food are enough to protect against diseases that may result from too little selenium. However, the food and drug administration recommends that adults take 55 mg of selenium a day and reported that selenium activates anti-oxidant enzymes and can boost the immune system and prevent cancer (Longtin, 2003; Broome, 2004). Arthur (1991) has proved the nutrient role of selenium in the active synthesis of thyroid hormone, where its deficiency leading to abnormality of thyroid functioning in human beings. Some reviews indicate that mortality from cancer, including lung colorectal and prostrate cancers (Russow et al., 1997; Patterson and Levander, 1997) and incidence of osteoarthritis (Kurz, 2002) is lower among the people with higher blood
selenium levels or intake. Selenium is also known to protect against toxic metals such as mercury and arsenic (Yoneda, 1997; Zeng, 2005). A review on brain research reported that selenium deficiency can lead to epileptic seizures and may even contribute to Parkinson's disease.

Selenium supplements for sheep were proved to be very effective in increasing the wool length and fiber diameter (Wilkins et al., 1982). Considerably more information is available for selenium accumulation and elimination in mammals (Daniels, 1996), avians (Heinz et al., 1990), and fish (Lemly, 1997).

Schwarz and Foltz (1957) demonstrated that trace elements of Selenium protect against liver necrosis in vitamin-E deficient rats and thus the nutritional essentiality of Selenium was established. Rotruck et al., (1973) reported the first known biological function of selenium as an important component of glutathione peroxidase, which can catalyze the reduction of peroxides that cause cellular damage. It is an established fact that meat quality is of increasing importance in commercial pork production. An attempt was made by Pehrson (1993), to improve meat quality and water holding capacity by adding antioxidants such as Vitamin – E and Selenium. Which is in combination with the enzyme, Glutathione peroxidase, prevented oxidative damage to cell membranes and improved their integrity, which ultimately lead to reduced moisture loss from the cell.
It was reported that the selenium is associated with proteins in animal tissues (Burk and Hill, 1993), and it nutritionally acts through its various selenoproteins to control the level of cellular hydrogen peroxides and redox tone of the cell organelle and D.N.A. It was also proved that the absence of selenoprotein of low molecular weight which can be compared to cytochrome, caused muscular dystrophy in selenium deficient sheep (M.P. Bansal and Parminder Kaur 2005). According to Clyburn et al., (2001), selenium supplementation improved the performance and meet quality in animals. It was reported that the supplementation of the diet with selenium normalizes the Drosophila life span by a process that may involve the newly identified proteins and experimentally proved that the number of eggs laid by Drosophila was reduced approximately in half in the chemically defined medium compared with the same medium supplemented with Selenium. (Javier et. al., 2001).

Very little information is available on the effects of selenium in arthropods. Particularly, insects are considered to be the critical components of most terrestrial and fresh water eco systems. They are key herbivores and recyclers and also become an important part of the food web for higher tropic levels. Despite these diverse roles, little is known about how some pollutants affect insects (Heliovera and Vaisemen, 1993). In particular, information on the effects of selenium on insect growth and survival is quite limited. Audas et al.(1995), estimated the curves of newly emerged Tenebrio molitor feed reared on
media containing different concentrations of selenium at different temperatures. An increased lethality was observed in controls when compared with the insects supplemented with diet containing selenium indicated the protective effect on survival for insects reared in media containing selenium. Selenium deficiency in the experimental models, coturnix and corcyra resulted in impaired mitochondrial substrate oxidations and lowered thiol levels (Knekt et al., 1998; Fleet, 1997). It is evident that the involvement of selenium in structural and functional efficiency of mitochondria (Rani and Lalitha 1996). Carla et al. (2006) investigated interactive effects of dietary selenium on growth and survival of house crickets Acheta domesticus L. and described improved survival and increased weight gain on the diet supplemented with selenium.

The above information clearly states that selenium is toxic to mammals especially at higher doses and beneficial at lower doses. But, the information on the interaction of selenium with different doses of selenium in insects has not available. Hence, in the present investigation, a study has been made on the toxicity of selenium at different doses and at different exposure periods in silkworm Bombyx mori L. The Study includes some biochemical and histological aspects in the organs of fat body, malpighian tubules and haemolymph of silkworms in relation to the accumulation of selenium. There are many reasons to select the above organs for studies. The fat body of an insect is an organ with multiple metabolic functions including
carbohydrates, lipids and nitrogenous compounds, the storage of glycogen, fatty acid synthesis and regulation of blood sugar and the synthesis of major haemolymph proteins (Dean et al. 1985; Keeley, 1985). A number of observations demonstrated that the insect fat body functional homology with mammalian liver (Abel et al. 1992; Sondergaard, 1993). Further, fat bodies played a role in the detoxification of metabolic environmental stimuli (Abel et al., 1992; Tae et al., 2002).

Insect malpighian (renal) tubules perform jobs analogous to the human kidney. They purify the blood (haemolymph) of waste materials, excrete and adjust primary urine, and thus play a major role in ion and water homeostasis (Gullan and Cranston 2000). Malpighian tubules are also major immune tissues, and they also detoxify many compounds, like human liver (Dow and Davies 2003).

The circulatory system in silkworm is an open system, haemolymph (blood) spends much of its time flowing freely within body cavities where it makes direct contact with all internal tissues and organs (Miller, 1985). About 90 per cent of haemolymph is plasma; a watery fluid which is clear, but some times greenish or yellowish in colour. Compared to vertebrate blood, it contains relatively high concentrations of aminoacids, proteins, sugars and inorganic ions (Mullins, 1985). Over wintering insects, often, sequester enough ribulose, trehalose or glycerol in the plasma to prevent it from
freezing during coldest winter (Worland and Block, 2003; Worland et al., 2004). The remaining 10 per cent of haemolymph volume is made up of various cell types, which are also called as haemocytes. They are involved in the clotting reaction, phagocytosis, and/or encapsulation of foreign bodies. These are free floating cells and play a role in the insect immune system (Schmid-Hempel, 2005). Therefore involvement and participation of the enzymes of the above organs in this new approach of toxicity of selenium stress is yet to be established in silkworms. Hence an attempt is made in this study to also estimate the carbohydrate, protein, accumulation and histological profiles in different tissues of silkworm Bombyx mori L. on exposure to lethal and sub lethal doses of selenium.