Chapter 1

Introduction
1. INTRODUCTION

The concept of recycling of natural resources is particularly of relevance to agricultural production, because the natural soil-plant-animal-soil recycling system is remarkably economic and effective in operating the principles of bio-processing and bio-conversion, the two aspects from the underlying idea of organic recycling. The concept of vermiculture bio-technology gives hope for healthy ecology and acts as a versatile natural bio-reactor. Earthworms which form one of the major soil macrofauna are of very important group of secondary decomposers. Sericulture is an agro-based cottage industry. The major activities in a sericulture industry are cultivation of food plants, rearing silkworms for the production of raw silk, reeling and other post cocoon processes such as twisting, dyeing, weaving, printing and finishing (Seshagiri and Rao, 2002). It produces significant quantities of secondary waste which may be processed in order to generate additional incomes. The complex substances can be converted in efficient nutrient pool with energy reserves as vermicompost through earthworms by regulating the moisture levels and mixing the ingredients in proper ratios.

1.1. Silk and sericulture

Silk is one of the oldest fibers known to man. Over thousands of years, it has become an inseparable part of the culture and tradition. Silk has been used by the Chinese since the 27th century BC and during the Roman empire, silk was sold for its weight in gold. It is an animal protein produced by certain insects to build their cocoon and webs. Silk is stronger than steel of the same thickness resisting breakage up to a weight of 4g per filament (Li and Hu, 2006). It is also lower in density than cotton, wool or nylon and highly moisture absorbent, being able to absorb moisture as much as a third of its own weight (Basker, 2006). Although many insects produce silk, only the filaments produced by the mulberry silk moth Bombyx mori and a few other sericiginous genus of family Saturniidae, are used in the commercial silk industry. Silk is a continuous filament -consisting of two proteins, secreted from salivary glands fibroin (73.5%) and a binding protein sericin.
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(22.28%) which cements the filaments together (Agrawal and Gopinathan, 1988). The silk filaments can be made free of sericin by putting the cocoons in hot water.

India is ranked as the second major raw silk producer in the world. It churns out value added products of economic importance. It is the only one cash crop in agriculture sector that gives returns within 30 days. It suits very well to all types of farmers and exceptionally for marginal and small land holders as it offers rich opportunities for enhancement of income and creates own family employment round the year (Singh and Benchamin, 2001).

1.2. Diversity of silk and silkworms.

The “Natural silk” consists of two main types i.e. mulberry silk produced by silkworm Bombyx mori insects belonging to the order Lepidoptera (Aruga, 1994), and the non mulberry silk like Muga, Tasar, and Eri collectively know as the Wild silk’ or “vanya” silk produced by wild silk worm species (Suryanarayana and Singh, 2003). The majority of the non mulberry silks produced are of eight different types (Kar, 2004).

Fig-1.1. Types of natural silk found in the world (Kar, 2004).

(1) Muga silk- produced by Antheraea assamensis of silkworm (2) Eri silk- produced by Philosamia sp. of silkworm (3) Tasar silk- produced by Antheraea sp.

1.2.1. Wild silk

India enjoys a unique distinction of being the only country in the world producing all varities of natural silk i.e. Mulberry, Tasar, Muga and Eri. The non-mulberry silkworms i.e. Muga, Tasar, and Eri are not fully tamed and the silk they produce is known as wild silk. These fibers although do not fit into the same group as mulberry silk owing to coarseness and mundane properties, can be blended with other yarns imparting a special texture and feel to the fabric. Of the three the Muga silk is limited to the state of Assam because of restricted food preference and steno-adaptation to specific geo climatic condition (Sharma and Kumar, 1998). However Eri and Tasar silkworms are wider in distribution in Eastern and Southern India because of their ability to survive under greater variations in temperature and rainfall. These phytophagus insects are cultured in most parts of the subcontinent as they can survive on several food plants. Tasar silkworm is a truly wild species as it cannot be reared indoors, whereas Eri worms are reared only indoors (Pandey *et al.*, 2005).

1.3. Eri culture

Being termed as “Poor man’s silk”, ericulture has all the necessary characteristics of a small scale cottage enterprise (Debraj *et al.*, 2003). Eri culture is distinctly divisible into two parts: - ericulture proper and Eri silk industry. The production of Eri silk from the silkworm *Philosamia ricini* by rearing on commercial scales is called Eriiculture. The Eri silk industry is the processing of cocoons and spinning cocoons into yarn (Chaudhury, 1982). It is a traditional vocation among the tribal people in North- Eastern region of India covering the states of Meghalaya,
Nagaland, Manipur and Arunachal Pradesh and it is also practiced in some pockets of Odisha, West Bengal, Bihar and Andhra Pradesh (Suryanarayana et al., 2003).

The Eri silkworm is polyphagous and feeds on several varieties of food plants like castor or Era (*Ricinus communis*) the primary food plant, Kesseru (*Heteropanax fragrans*), Payam (*Evodia flaxinifolia*), Tapioca (*Manihot utilissima*), Champa (*Plumeria acutifolia*), Bhotera (*Jatropha curcas*), Barkesseru (*Ailanthus glandulose*), Korha (*Sapium elloginifolium*), Bazrahan (*Zanthorylum yhesta*), The bow (*Hodgsonia heteroclitata*) and Papaya (*Carica papaya*) (Biswas and Das, 2001; Chakravorty and Neog, 2006). Nothing is waste in Eri culture as the by-products like castor shoots serve as fire wood and fuel, the left over leaves and excreta as manure and production of bio-gas (Reddy et al., 1998). The spun out pupae and used moths are used as poultry feed, and in the manufacturing of certain medicines and amino acids (Madan et al., 1989; Bose and Majumdar, 1990; Majhi et al., 1991; Deo et al., 1993; Singh and Suryanarayana, 2003).

1.3.1. Eri silkworm.

- **Systematic position**

  - **Kingdom** : Animalia
  - **Division** : Invertebrata
  - **Phylum** : Arthropoda
  - **Class** : Insecta
  - **Sub class** : Pterygota
  - **Order** : Lepidoptera.
  - **Super Family** : Bombycidae
  - **Family** : Saturniidae
  - **Genus** : *Philosamia*
  - **Species** : *ricini* (Hutton)
The lifecycle of Eri silkworm has five stages—egg, larva, pupa, cocoon, and moth (Fig. 1.2). The various stages in a lifecycle are (Ahmed, 1972)

- **Egg** - Eggs are ovoid, candid white measure about (1.5 x 1.0 mm) and weigh approximately 6 mg. The eggs hatch in 7-10 days.

- **Larva** - The larva on hatching measures about 7 x 1 mm and weight 1.5 mg. A fully grown (5th instar) measures larva measured about 7 x 1.5 cm and weigh 8 g. It is translucent and covered with white powdery substance. From the 3rd instar, body colour segregates into yellow, green, blue or white. Both spotted and unspotted larvae are found. The spots are of various types i.e. Single, double, zebra and semi zebra. The larva undergoes 4 molts during its larval period of 20-30 days.

- **Pupa** - The mature silkworm larva passes through a short transitory pre-pupa stage before becoming a pupa. The prominent morphological parts visible in this stage are a pair of large compound eyes, a pair of large antennae, fore and hind wings and the legs. Sex markings are prominent and it is much easier to determine the sex in the pupal stage than in the larval stage. After pupation the pupa is white in color, soft but gradually turns black to brown and pupal skin becomes hard. It measures about 2.8 x 1.5 cm and weighs about 2.6 g.

- **Cocoon** - The larva stops feeding at the end of the larval instars and starts secreting silk fluid through the spinneret. The secreting fluid in contact with air becomes silk filament and wrapped around the larva. The process of cocoon formation continues for about 3-4 days. The cocoons are elongated, wooly, soft, peduncle less, open mouthed and measure about 4 x 2.5 cm and weigh around 3 g.

- **Moth** - Moth emergence from cocoon takes place after about 2 weeks of cocoon formation. The moths exhibit distinct sexual dimorphism. The length of male is about 2.5 cm. and female is 3 cm. The colour of both
male and female is identical. The females are with bigger distended abdomen and bear narrow bipectinate antenna. After finding a suitable couple the moth undergoes copulation which lasts for several hours. After copulation the male dies and the cycle is repeated (Kar et al., 2005).

1.4. Tasar culture

Tasar silk, so named as "golden fiber" is a beautiful gift of nature produced by different Antheraea species (Sharma and Kumar, 1998). The tropical Tasar, Antheraea mylitta is indigenous to India. This biotic fauna has a diverse type of habitation in the central and southern plateau of India, particularly in the states of Bihar, Jharkhand, Madhya Pradesh, Chhatisgarh, Odisha and Maharashtra (Jolly, 1966). Tasar has nineteen different ecoraces and four mutant strains. Out of these ecoraces i.e. Modal, Nalia, Bogei, Daba and Sukinda are native to Odisha which is the second largest Tasar producing state in India (Dey et al., 2004). The tribals of the state take Tasar culture as additional income generating vocation supplementing their meager income.

Tasar is a silk reeled from the cocoons of wild silkworm Antheraea mylitta a polyphagus and semi domesticated insect mostly feeding on leaves of Terminalia tomentosa (Asan), Terminalia arjuna (Arjun) and Shorea robusta (Sal) (Sinha and Jolly, 1971). The major characteristics of the Indian Tasar silkworm is that it remains in a state of long pupal diapauses during summer and winter seasons (Jolly et al., 1970). Tasar is also different from other varieties of non-mulberry silk in respect to color, luster and strength (Pandey et al., 2005). Rearing of Tasar is an important part of applied biology that promotes conservation and sustainable utilization of natural resources. However Tasar culture produces a huge amount of waste containing such materials as larval excreta, leaf litter, dead larvae, moth and cocoons. Out of these the dead pupae after reeling are used presently for waste utilization (Shanna and Kumar, 1993).
1.4.1. Tasar silkworm

- **Systematic position**

  - **Kingdom**: Animalia
  - **Division**: Invertebrata
  - **Phylum**: Arthropoda
  - **Class**: Insecta
  - **Sub class**: Pterygota
  - **Order**: Lepidoptera
  - **Super Family**: Bombycidae
  - **Family**: Saturniidae
  - **Genus**: Antheraea
  - **Species**: mylitta (Drury)

- **Life cycle**

  The Tasar silkworm *Antheraea mylitta* is also completes its life cycle through 5 major stages such as egg, larva, pupa, cocoon, and moth.

  - **Egg** - The eggs are oval and dorso-ventrally flattened, with two brown lines running along the periphery and are coated with a gummy substance it measure about 1-3mm in length, 2.5mm in diameter. The incubation period of egg varies from 8-12 days depending upon seasonal temperature.

  - **Larva** - The newly hatched larva is dull, brownish yellow with a dark head. It measures about 7-9 mm in length and 8-9 mg in weight. After 2 days of hatching the body colour normally turns green and the head brown. This colour is retained throughout the larval period. At late fifth in star the larval size is about 13-15cm length and weight about 50-60 g. The Tasar silkworms are generally hardy, robust and possess tubercles, hairs, setae and many shining or metallic spot.
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- **Pupa** - The Tasar pupa looks dark brown in color and measures 3-4 cm in length, 1-2 cm in breadth and weights about 8-13 g.

- **Cocoon** - On reaching the end of fifth instars the larva stops feeding, prepare hammock by leaf and finally starts spinning. The larva takes 3-4 days to complete spinning. The cocoons are compact, oval, closed, and reliable with a long dark brown peduncle. The cocoon color is grey or yellow.

- **Moth** - The moth exhibits distinct sexual dimorphism. The females are bigger with distended abdomen and bear narrow bipectinate antenna. The females exhibit polymorphic color and are usually grey or yellow. The males are brown or brick red in color. The length of the male is around 3.5 to 4 cm and of female 4 to 4.5 cm. The copulation occurs after few hours of emergence and is usually continues for 10-12 hrs. But four hours of copulation is enough for effective fertilization, and hatchability.

1.5. Vermicomposting

Vermicomposting is a method of composting in which certain species of earthworms are used to enhance the process of waste conversion and production of better end product. (Gandhi et al., 1997). The science of vermicomposting took shape from mid-20th century and the first plant was started in Holland’s landing, Ontario, Canada (Appelhof, 1980). It is a mesophilic process that utilizes microorganisms and earthworms which are active at temperature range of 10 to 32°C. Earthworms play an important role in the initial phase of leaf litter fragmentation which in turn depends upon the type of litter present in the soil and the earthworm species (Standen, 1978).The process is faster than composting since the organic matter passes through the earthworm’s gut and ends up as excreta or cast, a major component in the compost thus produced. The cast is rich in microbes, plant growth regulating substances, and fortified with pest repellence attributes, (Suthar, 2006; Atiyeh et al., 2002). Being rich in macro and micro nutrients vermicompost is ideal organic manure for biomass production in agriculture and horticulture. Regarding the choice of earthworms attention of research workers were
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mostly focused on the feeding pattern of the worm which determines its growth, mortality and reproductive fitness (Dash et al., 1984). Vermicomposting technology involves three stages namely (i) preparation of compost beds (ii) collection of organic waste matter, screening and sorting of waste (iii) inoculation of earthworms and decomposition of waste. Depending on the suitability to the conditions vermicomposting methods such as bed, pit, heaping, tanks, cement rings etc have been adopted by different workers (Kale and Bano, 1988).

1.5.1. Organic waste for vermicomposting

Organic waste comes from plants and animals, which is biodegradable and is produced wherever there is human habitation. Vermicomposting can be done with any type of organic waste such as agriculture, sericulture, industrial, domestic and municipal origin.

• Agricultural waste

This is the waste which remains after the processing of crops in agriculture (e.g. maize stalks, rice husks, foliage etc.). Rice straw was used by Reddy and Ohkura (2004) for vermicomposting and they found that the compost prepared from it has higher nutrient content. Farmyard manure (FYM) from dairy farm mainly consists of discarded cattle feed, chopped fodder and animal excreta which can be used for vermicomposting (Suthar, 2009). Jowar straw (Sorghum vulgare) and millet straw (Pennisetum typhoides) are also rich in nutrients source for vermicomposting (Suthar, 2007). Agricultural waste from guar increases total nitrogen by vermicomposting (Suthar, 2006). Similarly agricultural residues such as sorghum straw, rice straw, dry leaves of crops and trees, pigeon pea stalks, groundnut husk, soybean residues, vegetable wastes, coconut fiber and sugar cane trash were used for vermicomposting by Nagavallemma et al., 2006. The agricultural residues and wastes such as tomato, potato, barley, sugarcane, sawdust, and soil mixed with cow manure rich in nutrients by vermicomposting (Ebadi et al., 2005). The farm garbage such as leaf litter, babul leaves, bean leaves, saw dust, paddy straw, wheat straw were converted into organic manure through vermicomposting by Indrajeet et al.(2010).
• **Sericultural waste**

In India another cottage industry is sericulture which produces 4000 tonnes of silk fiber waste, 16 lakh tonnes of mulberry twigs, 10 lakh tonnes of the left over bed refuses of mulberry leaf bits, 5 tonnes of larval litter, 0.5 lakh tonnes of reeled pupae and 750 tonnes of silk moth biomass per year (Gunathilagaraj and Ravignanam, 1996). This waste can be recycled by vermicomposting. Mulberry culture waste used in vermicomposting and the compost can be applied in the field to increase in the leaf yield of mulberry plants (Bhogesha et al., 1997; Gunathilagaraj and Ravignanum, 1996). Similarly sericultural waste (mulberry culture waste) was used by Chaudhury et al., 1993 and found that it was higher nutrient content than farm yard manure.

• **Industrial waste**

Several industries such as paper mill, cotton mill, rice mill, sugar factory, food processing plant, brewery, oil processing plant and soap manufacturing units generate large amount of organic and biodegradable waste (Kastner and Mahro, 1996; Lalande, et al., 2003; Kaushik and Garg, 2004). Industrial waste from coffee plants can be processed through vermicomposting (Orozco et al., 1996). The waste from sugar, aromatic oil, and distillery industries produce quality manure through vermicomposting (Seenappa and Kale, 1993; Elvira et al., 1996). Textile industries produce various types of waste and that can be enriched with NPK by vermicomposting Garg et al. (2006). Industrially produce woodchips when decomposed by *Eisenia fetida* produce quality manure (Maboeta and Rensburg, 2003). The food industry wastes and bagasse from sugarcane factories are also converted into quality compost through vermicomposting (Nagavallemma, 2006). Coir industry produce a huge amount of waste that can be recycled through vermicomposting (Vijaya et al., 2008).

• **Domestic or household waste**

This type of waste is usually made up of food scraps, either cooked or uncooked, and garden waste such as grass cuttings or trimmings from bushes and hedges. Waste in large amount is generated at institutions such as schools, hotels and
restaurants and the vermicompost out of the waste is rich in nutrients (Suthar, 2007). The vegetable waste from market was used in vermicomposting and shown to be effective for plant growth (Chauhan et al., 2010). The domestic refuse can be converted into nutrient rich compost by epigeic earthworms *Eisenia foetida* and *Eudrilus eugeniae* (Kale and Bano, 1988). Kitchen waste such as vegetable skin (potato and onion), leaves of cabbage, parts of cauliflower and carrots, fruit skin (banana), egg shells, drained liquid of boiled rice can be converted into quality compost by earthworms (Chaudhuri, 2000).

- **Municipal waste**

  Municipal waste mainly consists of street sweepings, landscape and tree trimming, and general waste from parks, beaches, drain sludge, sewage and other recreational areas which can be recycled into quality compost. The solid waste from municipal and urban area provides high nutrient rich material for vermicomposting (Lodha, 2007; Pattnaik and Reddy, 2010). Solid paper pulp mill waste from municipal area was converted into quality compost with anecic earthworm *Lampito maruitti* (Elvira et al., 1996). The sludge from paper mill and dairy industry was also used for converting into nutrient rich compost by Elvira et al. (1998). The paper mill sludge can be converted into nutrient rich compost through vermicomposting (Kaviraj and Sharma, 2003; Umanaheswari and Vijayalakshmi, 2003; Lalande et al., 2003; Gajalakshmi and Abbasi, 2004).

### 1.6. Earthworms in vermicomposting

Earthworms are aerators, mixer of soil, chemical degrader of complex compounds and play a significant role in decomposing organic waste material and mineral cycling (Kale and Bano, 1988). They can consume and degrade all kinds of organic matter through digestive enzymes and transport organic and inorganic material in order to enrich soil (Dash and Patra, 1979). Nutrient composition of the vermicompost varies with the species of earthworm. Therefore the selection of earthworm is an important step in the vermicomposting protocol (Appelhof, 1980). There are 4400 earthworm species which are divided into 3 major groups depending upon their ecological niche i.e. epigeic or surface soil dweller, anecic or sub-surface...
soil dweller and endogeic or bottom soil dweller (Bouche, 1977). The epigeic earthworms are mostly used in vermicomposting because of their unique features in life cycle. These worms inhabit in soil with high percentage of organic matter, have higher fecundity rate, low incubation period and need least time for maturity (Dash, and Senapati, 1982). For vermicomposting most used species of earthworms are *Perionyx excavatus* (Indian), *Eisenia foetida* (European) and *Eudrilus eugeniae* (African) (Kale and Dinesh, 2005). Among these three species *Perionyx excavatus* and *Eisenia foetida* are commonly used in India for composting.

1.6.1. *Perionyx excavatus*

*Perionyx excavatus* is a tropical species distributed in India and south East Asia. In India the species is common in Odisha, Assam, Arunachal Pradesh, West Bengal, Maharashtra, Andaman and Nicobar Islands (Gates, 1972). *Perionyx excavatus* has an iridescent blue or violet shine on its skin clearly visible under bright light it has an impressive growth and reproductive rate far in excess of the other species. It is used for vermicomposting process in many parts of world due to its high potential in the decomposition of organic matter (Kale *et al.*, 1982). The systematic position of *Perionyx excavatus* is

- **Kingdom**: Animalia
- **Phylum**: Annelida
- **Class**: Oligochaeta
- **Order**: Opisthopora
- **Family**: Megascolicidae
- **Genus**: Perionyx
- **Species**: excavatus (Perrier)

1.6.2. *Eisenia foetida*

*Eisenia foetida*, another species of earthworm adapted to inhabit decaying organic material. They thrive in rotting vegetation, compost, and manure. They are native to Europe, but have been introduced to every other continent
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(Mitchell and Edwards, 1997; Ndegwa and Thompson, 2000). The worm survives at a wide range of temperature (4-28°C). In summer it has high foraging activity whereas in winter the worm is sluggish and its metabolism is retarded. It comes to the surface during rainy season because the worm can not respire as its burrows are flooded. The systematic position of *Eisenia foetida* is

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<td><em>Eisenia</em></td>
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<td>Species</td>
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1. 7. Soil enzymes

Enzymes play an important role in soil system catalyzing several reactions during the decomposition of organic matter (Burns, 1982; Sinsabaugh *et al.*, 1991). These are essential for micro-organisms, for stabilization of soil structure, and nutrient cycling (Dick *et al.*, 1994). Each type of soil contains a specific group of enzymes which are dependent on the microorganisms, physico-chemical and biochemical properties of the soil (McLaren, 1975). Also the enzyme level varies with the soil type, organic matter content, microorganisms and their biological processes (Kiss *et al.*, 1978). Thus the assay of enzymes in soil is an important index for determining the rate of breakdown of complex organic matter and the release of the mineral elements (Burns, 1982).

These soil enzymes mainly catalyze metabolism of carbohydrates, nitrogen and phosphorous containing compounds and several oxidation reduction processes (Bolton *et al.*, 1985). The enzymes which are exuded from the cells of soil organisms are known as extracellular enzymes. These hydrolyze large polymeric molecules like starch, cellulose, hemicelluloses, lignin and pectin. Intracellular enzymes are that act inside cells and are responsible for catalyzing the several of
reactions that occur in metabolic pathways such as glycolysis in the mitochondria and in the photosynthetic pathway in the chloroplast (Kiss et al., 1978).

There are many enzymes responsible for various activities in soil; among these are amylases, cellulases, invertase, dehydrogenases, and phosphatases responsible for various decompositions in soil (Sajjad et al., 2002). Sources of soil enzymes include living and dead microbes, plant roots and residues, and soil animal. Extracellular enzymes stabilized in the soil matrix accumulate or form complexes with organic matter or humus, clay, and humus-clay complex, but are no longer associated with viable cells. In an average 40 to 60% of enzyme activity comes from the stabilized enzymes since it does not necessarily correlate with microbial biomass or respiration (Bolton et al., 1985). Thus the enzymes in complex form are efficiently functional in soil for long term microbial activity (Kiss et al., 1978).

- **Amylase**

Amylase is a digestive enzyme classified as a saccharidase (that cleaves polysaccharides) that catalyze hydrolysis of alpha-1, 4-glycosidic linkages of polysaccharides to yield dextrins, oligosaccharides, maltose and D-glucose. During vermicomposting amylases are the major group of enzymes secreted by the microbes (Parthasarathi and Ranganathan, 2000). They are mainly of two types, α (α-1, 4-glucan-4-glucanohydrolase) and β (α-1, 4-glucan maltohydrolase). The α-amylases which mainly are products of fungi and bacteria hydrolyze starch to form dextrin and a small quantity of maltose and glucose (Thoma et al., 1971). The β-amylase on the other hand breaks down starch into maltose and a small quantity of glucose and dextrin. The activity of these amylases is influenced by many factors such as types of vegetation, environment and soil type. (Pancholy and Rice, 1973; Ross, 1975)

- **Cellulase**

Cellulose the principal constituent of the cell wall of plants is an indigestible-polysaccharide and is the most abundant organic matter in the biosphere, comprising almost 50% of the biomass synthesized by photosynthesis. The cellulose in the soil is derived mainly from plants debris and limited amount from fungi and bacteria (Richmond, 1991). The enzyme cellulase has cellulolytic
activity, i.e. that it attacks the $\beta$-D-1, 4-glycosidic bonds of cellulose and breaks down cellulose into $\beta$-glucose, cellobiose and high molecular weight oligosaccharides (White, 1982; Sajjad et al., 2002).

- **Invertase**

  The systematic name for invertase is $\beta$-fructofuranosidase and it usually refers to enzyme from either fungal, bacterial or a plant source that splits the bond between the glucose and fructose by hydrolysis (Sajjad et al., 2002). Invertase belongs to a class of enzymes known as glycosidase. Optimum activity of the enzyme was found to be in broad zone from pH 4 to 5.5.

- **Phosphatases**

  Phosphatases (orthophosphoric monoester phosphohydrolases) are group of enzymes actively engaged in removing phosphate from various organic molecules and thus enriching the soil (Sajjad et al., 2002). Phosphatases are of two types basing on their pH dependence. Alkaline phosphatase as the name suggests is a hydrolyzing enzyme responsible for removing phosphate group in an alkaline environment, from many types of phosphate bearing molecules like nucleotides, proteins, and alkaloids (Bayon and Binet, 2005). Acid phosphatase on the other hand performs the similar function in an acidic environment or lower soil pH. In soil ecosystem, acid phosphatases are known to play critical role in phosphorus cycle and are good indicators of soil fertility (Dick et al., 2000).

- **Dehydrogenase**

  Dehydrogenase is a universal enzyme that belongs to class of oxidoreductase, produced by all organisms and linked with respiratory process (Bolton et al., 1985). It is an intracellular enzyme that catalyzes oxidation and reduction reaction in organic compounds to generate energy. During microbial respiration it oxidizes soil organic matter by transferring protons and electrons from substrates to acceptors (Garcia et al., 1997). Dehydrogenase is considered to exist as an integral part of intact cell. It is used as index of biological activity of soil (Burns, 1978). Studies on the activities of dehydrogenase enzyme in the soil is important as
it gives indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility (Bolton et al., 1985).

1.8. Fungi in vermicomposting

Fungi are saprophytic organisms which can survive for long periods of water deficit in the soil. They are found wherever there is carbon-rich organic matter like trees in a forest, leaf litter on the surface of orchard soils, or plant roots. Fungi in soil systems perform important services related to water dynamics, nutrient cycling, and disease suppression (Ritz and Young, 2004). Along with bacteria, fungi are important as decomposers in the soil food web. They convert complex organic matter into simple forms into that other organisms can use (Hoorman, 2011). Fungal hyphae physically bind soil particles together, creating stable aggregates that help increase water infiltration and soil water holding capacity. Fungi are capable of degrading cellulose, proteins and lignin, some of which are highly resistant to the process of degradation. They play an important role in immobilizing and retaining nutrients in the soil (Rabia et al., 2007). Apart from the nitrogen fixation, fungi have the ability to produce growth promoting substances which favor better growth of crops. Some heterotrophic fungi are known to have ability to solublize phosphorous from the inorganic sources (EL-Azouni, 2008). Fungi use many carbon sources, mainly lignocelluloses polymers and can survive in extreme conditions. They also plays important role in soil aggregation and in the formation of humus. In vermicomposting the fungal varieties which contribute the process are mainly of two types.

- Decomposers

The saprophytic fungi convert dead organic material into fungal biomass, carbon dioxide (CO$_2$), and small molecules, such as organic acids. These fungi generally use complex substrates, such as the cellulose and lignin, in wood, and are essential in decomposing the carbon ring structures in some pollutants. Fungi are important for immobilizing, or retaining, nutrients in the soil. In addition, many of the secondary metabolites of fungi are organic acids, so they help increase the
accumulation of humic-acid rich organic matter that is resistant to degradation and may stay in the soil for hundreds of years.

- **Mutualists**

  There are a group of fungi which colonize plant roots (mycorrhizal fungi) and help plants to obtain nutrients at that level. In exchange for carbon from the plant, mycorrhizal fungi help solublize phosphorus and bring soil nutrients (phosphorus, nitrogen, micronutrients, and perhaps water) to the plant (Rabia et al., 2007). One major group of mycorrhizae, the Ectomycorrhiza, grows on the surface layers of the roots and is commonly associated with trees. The second major groups of mycorrhizae are the endomycorrhizae that grow within the root cells and are commonly associated with grasses, row crops, vegetables, and shrubs. Arbuscular mycorrhizal (AM) fungi are a type of endomycorrhizal fungi. Number of soil fungi forms mycorrhizal association with the roots of higher plants (symbiotic association of a fungus with the roots of a higher plant) and helps in mobilization of soil phosphorus and nitrogen e.g. Glomus, Gigaspora, Aculospora, (Endomycorrhiza) and Amanita, Boletus, Entoloma, Lactarius (Ectomycorrhiza) (Hoorman, 2011).

**1.9. Literature Review**

**1.9.1. Vermicomposting**

In vermicomposting the earthworms decompose organic waste and release nutrients like nitrogen, potassium, phosphorous and calcium and convert them to soluble and available forms (Benitez et al., 2005; Garg and Kaushik, 2005; Suthar, 2006). Vermicomposting through different species of earthworm has been studied by several researchers and it has been well established that epigeic forms of earthworms can hasten the composting process to a significant extent with production of better vermicompost (Kale et al., 1982; Edwards et al., 1998; Ndegwa and Thompson, 2001). The epigeic earthworm *Eisenia fetida* is a suitable species for management of waste which is utilized successfully in vermicomposting (Garg and Kaushik, 2003; Garg and Kaushik, 2004). Lavelle (1996) investigated that in tropical soils an adult earthworm in an average ingests three times more than its body weight. In Indian pasture soils, *Drawida sp.* such as *D. willisi* and *D. calebi*
ingest about twice the body weight while other species like *Lampito mauritii* and *Octochaetona surensis* ingest about 2 to 3 times the body weight per day (Dash *et al.*, 1986).

Guild (1952) reported that the burrowing activity of earthworms increase soil aeration and drain water 4 to 10 times faster. A case study by Satchell (1967) and Dash (1999) indicated that the nitrogen added to soil by a single adult dead *Lampito mauritii* can yield as much as 30 mg nitrogen and a population of 2 million earthworms per hectar in tropical grassland could yield the equivalent of about 60 kg nitrogen per hectar.

Studies of vermicomposting by most workers concerned the role of earthworms in nutrient cycling, waste land reclamation, crop productivity (Chaudhuri *et al.*, 2003; Sinha *et al.*, 2003; Tripathi and Bhardwaj, 2004). Vermicomposting is a stabilization of organic material involving the joint action of earthworms and microorganisms. Although microbes are responsible for the biochemical degradation of organic matter, earthworms are the important derivers of the process, conditioning the substrate and altering the biological activity (Aira *et al.*, 2002). Tiunov and Scheu (2000); Dominguez *et al.* (2002) have worked on earthworm micro flora interactions and found that worms utilize micro fungi and soil nematodes during its processing of food in the gut. Dash *et al.* (1984) reported that the grazing activities of earthworm over microorganisms prevent ageing and enhance growth of microorganisms in soil. Dash (1987) have found that earthworms feed mainly on non-parasitic nematodes and Dominguez *et al.* (2002) showed significant reduction in the numbers of bacterivore nematodes as sewage sludge passed in earthworm gut.

Edwards and Heath (1963) reported that earthworms play important role in the initial process of leaf litter fragmentation. The rate of breakdown of litter depends upon the type of litter (Kiss *et al.*, 1978). Several workers like Kurcheva (1960), Edwards and Heath (1963), Witkamp and Crossley (1966) have measured the rate of decomposition through litter bag studies. Dash and Cragg (1972), Standen (1978) have established the role of soil fauna in the breaking down of litter and decomposition process. In temperate deciduous woodland studies in Europe
(Satchell, 1967) showed that leaf fall is around 3 ton / ha / year and earthworms consume about 27 g of leaf litter per day. Thus the entire annual leaf fall would be consumed by the worms in three months period. Several studies on analysis of the gut of earthworms showed the presence of a wide variety of enzymes such as amylase, cellulase, phosphatases, and proteases in it (Loh et al., 2005; Sen and Chandra, 2007; Pramanik et al., 2007; Suthar, 2007; Padmavathiamma et al., 2008).

1.9. 2. Soil nutrients

Nutrients (both micro and macro nutrients) are important for plant growth. During vermicomposting the nutrients are released in the form of compost. Nitrogen, phosphorus, potassium and sodium are the primary microelements where as calcium, magnesium, sulfur etc. are the secondary microelements (Edward, 1998). Vermicompost, especially from animal waste sources, usually contained these mineral elements which promote biological activity and improves plant growth. (Edwards and Burrows, 1988; Edward, 1998; Arancon et al., 2004; Atiyeh and Dominguez, 2000). These are mineralized into the soil and this mineralization process has been investigated by various workers. Senesi et al. (1992), Kale et al. (1992), Orozco et al. (1996) reported that vermicompost is a superior biofertilizer for its high NPK, Ca, Mg, water holding capacity and higher ionic content.

Many workers have used several waste products for vermicomposting. Guerra-Rodriguez et al., 2000 worked on toxic poultry manure and converted it into nontoxic organic waste through vermicomposting. Indrajeet et al. (2010), Kale and Bano (1988) have converted agro waste and domestic refuse into nutrient rich compost by epigeic earthworms Eisenia foetida and Eudrilus eugeniae. Vermicomposting of crop residues and cattle dung results in a significant reduction in C/N ratio and increase in N and microbial activity by the use of the epigeic earthworm Eudrilus eugeniae (Suthar, 2007). Vermicomposting of pig-manure produces a high nutrient humus rich, odour free vermicast (Atiyeh et al., 2001; Aira et al., 2007). According to Chauhan et al. (2010), Raphael and Velmourougane (2011) working on the vegetables waste and coffee pulp reported that the pH of the soil declines by vermicomposting of the waste. Garg et al., 2006 have shown that vermicomposting of the livestock excreta using Eisenia foetida
lowers the soil pH. Similar finding was reported by Atiyeh et al. (2001) with pig manure. Indrajeet et al. (2010) reported that the pH of the vermicompost prepared from farm garbage reduces the pH to near neutral.

Gandhi et al. (1997) have shown that household waste is converted into vermicompost within 30 days and reduces the C/N ratio and retains more N than the traditional methods composting. Integrating composting and vermicomposting methods for the treatment of biosolids and the effects on C/N ratio was studied by several workers like Ndegwa and Thompson (2001), Reddy and Ohkura (2004), Tripathi and Bhardwaj (2004). Vermicompost with three earthworm species *Eisenia fetida*, *Eudrilus eugenae* and *Perionyx excavatus* of vegetable wastes with cow dung has higher NPK content and lower C, C/N, and C/P ratio (Chauhan et al., 2010). Businelli et al. (1984) by vermicomposting reported higher nutritional values from cattle and horse manure mixture compared to that of the municipal waste.

Orozco et al. (1996) reported that the coffee pulp increases the availability of nutrients such as phosphorus, calcium and magnesium, after processing by *Eisenia fetida*. Reddy and Ohkura (2004) showed that rice-straw was higher in nutrients when decomposed by the species *Perionyx excavatus*. Suthar (2006) conclude that an increase in total N, available P and exchangeable K content of the vermicompost by farm yard manure (FYM) mixed with cattle dung. Agricultural waste, horticultural waste, animal waste, silkworm litter, plant biomass, weeds, kitchen waste, city refuge and other biodegradable materials can be converted to nutrient rich compost by vermicomposting (Kaushik and Garg, 2004). Kurien and Ramasamy (2006) found that vermicomposting of Taro (*Colocasia esculenta*) increased nutrients in the soil. Suthar (2007) reported higher nutrient content of domestic waste when decomposed by earthworm *Perionyx sansibaricus*. Similarly vermicomposting of municipal solid waste, urban waste enrich the soil with high nutrient content as reported by Lodha, (2007), Pattnaik and Reddy (2010). Vermicomposting of mulberry waste using *Perionyx excavatus* considerably increased nitrogen and phosphorus content in soil (Gunathilagaraj and Ravignanam, 1996). Das et al. (2003) showed that the K, Mn, Zn, Fe content and micro flora activity of mulberry leaves were higher than farm yard manure (FYM). Working on
similar lines, Venugopal et al. (2010) reported higher nutrient content in vermicompost of coir and sericulture waste as compared to cow dung.

Several workers like Elvira et al. (1998), Umamaheswari and Vijayalaxmi (2003), Gajalakshmi and Abbasi (2004), Elvira et al. (1996) have used paper mill sludge and converted it into nutrient rich compost using African earthworm species Eudrilus eugeniae (Kinberg). Similarly Kaviraj and Sharma (2003) reported that humification rate increased significantly when the paper (pulp) mill sludge was decomposed by the earthworm Eisenia andrei. Seenappa and Kale (1993), Elvira et al. (1996) concluded that the waste from sugar factory, aromatic oil industries, and distillery also produces quality manure through vermicomposting. Garg et al. (2006) showed that the waste water sludge of textile mill and the bio gas slurry can enrich the soil with NPK by vermicomposting. Garg and Kaushik (2005) reported that vermistabilization by Eisenia foetida of textile mill sludge spiked with poultry dropping increased the NPK content and decreased the C/N ratio. Maboeta and Rensburg (2003) found that industrially produced woodchips and sewage sludge when decomposed by Eisenia foetida produced quality manure. Guar gum industrial waste also can be converted into good quality manure through vermicomposting as reported by Suthar (2006).

1.9.3. Soil enzymes in vermicomposting

Soil enzymes play a key role in nutrient cycling (Joachim et al., 2008) and the level of enzymes in the soil vary according to the soil type, organic matter, composition and activity of soil organisms (Burns, 1982). Enzyme activity acts as indicator of the process of composting by the earthworms (Benitez et al., 1999). According to Schaller (2009) enzymes like amylase, cellulase, phosphatase, and urease are the indices of soil fertility.

In soil, extracellular enzymes like amylase and invertase exhibit variations in activity in compost and vermicompost (Devi et al., 2009). Parthasarathi and Ranganathan (2000) have reported that amylase and invertase activity during vermicomposting in worm cast of pressmud and filter cake declined steadily with the aging of the cast. Singaram and Kamalakumari (1995) have shown that amendment of the soil by farm yard manure (FYM) resulted in significant increase
in the amylase activity. Similarly invertase activity was enhanced during decomposition of plants residues of wheat, maize and sesbania (Sajjad et al., 2002). Hubbe et al. (2010) have reported conversion of cellulosic biomass and organic waste into nutrient rich high value compost through the action of cellulase. Paper mill sludge which is high in cellulosic content is converted into nitrogen rich material by enzymatic cellulolysis during vermicomposting (Charest et al., 2004; Gea et al., 2005; Monte et al., 2009). Several researchers have worked on decomposition of various cellulosic materials and cellulase activity through the process of vermicomposting. (Rao et al., 2007; Sung and Ritter, 2008), Lignocellulose from different sources such as paper (Ekinci et al., 2000; Rao et al., 2007), cardboard (Francou et al., 2008; Saludes et al., 2008), pulp mill solids (Levy and Taylor, 2003), saw dust (Tang et al., 2007) sugarcane residue (Boopathy et al., 2001), olive oil waste, brewery waste, cotton waste (Garcia-Gomez et al., 2005), leaves, wood saving and peat (Eklind and Kirchmann, 2000), cocoa husks (Rao et al., 2007), oil palm residue (Saletes et al., 2004), wood chips (Maboeta and Rensburg, 2003; Suzuki et al., 2004).

One of the major nutrients obtained by the vermicomposting is phosphate. It in turn is related to the enzyme phosphatase which releases organic phosphate to the soil. The phosphatase activity was shown to be significantly increased in municipal solid waste compost than the control. (Garcia-Gil et al., 2002; Gaind and Lata, 2004; Satchell and Martin, 1984; Ranganathan and Vinotha, 1998; Vinotha et al., 2000) have reported that the earthworms are responsible for addition of alkaline phosphatases, produced in the worm gut and are then excreted through cast. Compost prepared from the sewage sludge by using the earthworm L. terrestris has a significant phosphates activity by increasing the phosphorus content in the compost (Bayon and Binet, 2005). The acid and alkaline phosphatase activity of the vermicompost is significantly higher compared to the compost prepared by conventional method (Zachariah and Chhonkar, 2004).

The enzyme dehydrogenase is known to be linked with the microbial activity for the initial breakdown of organic matter (Ross, 1971). Ross (1970) reported that dehydrogenase being an intracellular enzyme is more dependent on the metabolic state of the soil rather than on the free enzyme. In the rice field,
dehydrogenase activity increased significantly by applying agricultural and farm
yard manure (Sriramachandransekhara et al., 1997; Gaind and Lata, 2004). Garcia
et al., 1997 reported that the dehydrogenase activity can be an index of microbial
action in soil systems. Forster et al. (1993) showed that soil dehydrogenase activity
increased significantly in pig manure vermicompost more than the normal compost.

1.9.4. Fungi in vermicomposting

An important aspect in vermicomposting is the relationship that
earthworms establish with microorganisms in decomposing organic matter and they
compete for the same resource pool (Scheu and Falca, 2000; Tiunov and Scheu,
2004). Vermicompost has large particulate surface area that provides many sites for
microbial activity and for the strong retention of nutrients as reported by Scheu
(1987). Vermicomposts are rich in microbial populations, particularly fungi, bacteria
and actinomycetes (Kavitha et al. 2011; Edwards, 1998). Increased microbial
activity resulted in increased mineralization of micro and macro nutrients in the cast
(Parthasarathi, 2006). Temperature is the most important factor during
decomposition. The mesophilic bacteria and fungi are dominant in the initial period
of vermicomposting which is most favorable for their growth (Finstein and Morris,
1975).

Many researchers have studied the earthworms association with
microflora during decomposition (Nijahwan and Kanwar, 1952; Khambata and Bhat,
1957; Bhat et al. 1960). From an experiment Dash et al. (1986) established that
earthworms digest eight different species of microfungi in different region of the gut.
Many other researchers have reported the earthworm - microflora interactions and
found that worms utilize micro fungi and soil nematodes as food (Tiunov and Scheu,
2000, Dominguez et al., 2002). Dash et al. (1984) reported that maximum numbers
of micro fungal species occur in the fore-gut, gradually decreasing in the mid-gut
and hind-gut with minimum number occurring in freshly laid cast. The number of
microfungi in the fore gut of earthworms is highest, but gradually decrease in mid
 gut to hind gut and the least is found in freshly laid cast (Dash et al., 1984). In
vermicompost there is relative increase in microbial population, activities of soil
enzymes and nutrients (both micro and macro) compared to conventional
composting without earthworms (Satchell and Martin, 1984; Edwards and Bohlen, 1996).

Earlier studies by Parthasarathi and Ranganathan (2000), Beffa et al. (1998) have established that the foul smell of the pressmud can be removed by vermicomposting with *L. mauritii* and *E. eugeniae*. It also significantly enhances nutrients, soil enzyme activity and microbial population. Pressmud vermicast was found to be the 'hot spot' of fungi, bacteria which subsequently decrease by aging of the casts (Scheu, 1987; Parthasarathi and Ranganathan, 2001). Earthworms stabilize organic residues and reduce pathogenic bacteria and affect fungal communities by influencing spore germination and creating micro sites for development of fungus (Brown 1995; Tiunov and Scheu, 2000). Fungi also degrade complex polymers such as polyaromatic compounds or plastics (Kastner and Mahro, 1996; Eggen and Sveum, 1999; Minussi et al., 2001; Ashraf and Ali, 2006). Rabia et al. (2007) have found that the cellulolytic fungi, such as *Aspergillus*, *Trichoderma*, *Penicillium* and *Trichurus* accelerate efficient composting of dry crop wastes with high C/N ratio and reduce the period of composting.
1.10. Objectives

From the review of literature it is seen that the nutrient status is high in all types of organic waste irrespective of whether it comes from agriculture, domestic or municipal sources. However each waste has a unique physico-chemical composition and bioconversion efficiency. The reports show that although some of the waste materials have been used for vermicomposting studies, there is hardly any information regarding utilization of waste from non-mulberry silk worm culture. Hence the present piece of work intends to focus on the conversion of the byproducts of Eri and Tasar culture into quality manure through vermicomposting and its evaluation as the biofertilizing agent. Other important consideration for pursuing this work is its societal relevance. Sericulture in the western part of Odisha is practiced by small and marginal farmers as a means for augmenting their income. Therefore the conclusion of this work can be extended to the small farmers having sericulture as additional vocation and also will be helpful for the disposal of the waste.

Before embarking on the actual experimentation, it was pertinent to select suitable earthworm species for vermicomposting. Mostly the epigeic earthworms are chosen for composting as they have many metabolic and physiological features suited to the decomposition process. In India the epigeic exotic earthworm species *Eisenia fetida* which adapts to all types of climates and *Perionyx excavatus* a native worm in most parts of the country are used extensively for vermicomposting experimentation. In the present work the earthworm species selected were *Eisenia fetida* and *Perionyx excavatus*. For analysis of the dynamic changes in decomposition and characterization of the final product we have chosen such components as pH, carbon, nitrogen and other nutrients. Similarly for understanding the process of decomposition, enzymes the real converters of the complex organic molecules to assimilable form (both extra and intracellular origin) were taken for analytical studies. Also the enumeration of fungi was chosen for understanding the conversion of organic waste and augmentation of nutrition in the composting process.
Introduction

With this background information the objectives of the present work were.

1. To culture the Eri silk worm (*Philosamia ricini*) in laboratory using standard procedure and to prepare vermicompost using sericulture waste (Eri and Tasar) by standardized methods.

2. To analyze the compost on different days interval and biochemical characterization using standard methods.
   
   i. To measure the pH of the compost
   
   ii. To measure the carbon, nitrogen and C/N ratio of the compost
   
   iii. To measure the sodium and potassium content of the compost
   
   iv. To measure the phosphorus and C/P ratio of the compost
   
   v. To assay amylase, cellulase and invertase activity of the compost
   
   vi. To assay the activity of acid phosphatase of the compost soil
   
   vii. To measure the activity of dehydrogenase of the compost soil

3. To enumerate the fungal colonies on different days of composting.
Fig- 1.2. Life cycle of Eri silkworm (*Philosamia ricini*)
Fig- 1.3. Life cycle of Tasar silkworm (*Antheraea mylitta*)