CHAPTER 2
REVIEW OF LITERATURE

2.1 Origin of silk

According to Chinese myth, the origin of silk can be traced back to ancient China where it has a fascinating history. The story from the 27th century BC claims that the finding of silk occurred accidentally by Queen His-Ling-Shih, wife of the third emperor of China, Huang-Ti, in 2500 BC. It claimed that one day, while Empress was drinking tea, a cocoon accidentally fell into her cup of tea. The filament of the cocoon started to loosen and unraveled into a delicate thread. The Empress then had the idea to weave the string, and she eventually mastered the craft of sericulture. This marked the beginning of the silk industry in China.

The Chinese kept the existence of silk a mystery from the rest of the world for a very long time. The Silk Road, the lucrative trade routes connecting the East and the West world was only established in the latter part of the first millennium BC and it played a major role in spreading silk beyond China. The Silk Road connected the Mediterranean region with North Africa and Europe. As the silk industry started spreading, various nations adopted sericulture. Initially, nations like India and Japan learned about sericulture and quickly began competing with the eastern monopoly on silk production. By the 6^{th} century, silkworm eggs secretly reached Byzantium, marking the beginning of European silk production. The silk production eventually expanded beyond Asia despite the secrecy. The Arab traders played key role in introducing silk to Middle East and the Mediterranean. And gradually, sericulture has become one of the most important cottage industries in several countries like China, Korea, Brazil, Russia, Italy, and France. Techniques for silk production continued to evolve, today, China and India are the two main producers, together manufacturing more than 60% of the world production each year.

2.2 Overview of Sericulture in India

Sericulture in India is a centuries old traditional practice that involves the raising of food plants for silkworm, rearing of silkworm for production of cocoons, reeling and spinning of cocoon for production of yarn for value-added benefits such as processing and weaving. India has the distinction of producing all the four commercial types of silk i.e. Mulberry silk, Tasar silk, Eri silk and Muga silk which are produced by *B. mori, A. proylei, S. ricini* and *A.*

assama respectively (Babulal et al., 2017). The history of eri silk in India is thought to have originated in Brahmaputra valley of Assam and adjoining foothills of Sub-Himalayan best which can be traced back to the Vedic literature (1600 BC ago). The eri silkworm has derived both its scientific and its vernacular name from its attachment to the castor-oil plant, called 'eri' or 'era' in Assamese and 'indi' in Bodo (Chakravorty et al., 2010)

According to Singh and Benchamin (2001) the eri culture is practiced by the tribal inhabitants of Kokrajhar, Karbi Anglong, Goalpara, Kamrup, North Lakhimpur, Dhemaji, Nagaon, Golaghat, Jorhat, Morigaon etc. in Assam and also in several districts of Meghalaya, Nagaland, Mizoram, Manipur and Arunachal Pradesh. Utilization of silkworm as food is practiced in many countries of the world. For the tribal community of Northeastern India, the chrysalis (pupa) of eri is regarded as a delicacy and the cocoon is more or less a by-product (Sarmah et al., 2011).

Among all the silks, eri silk is becoming popular in recent years. India is the largest eri silk producer in the world, accounting for 96% of the total silk production. Ericulture is indigenous to Northeastern part of India, which is extremely rich in Seri-biodiversity. Eri culture is extensively practiced in Assam, Arunachal Pradesh, Manipur, Meghalaya, and Nagaland. Gradually the demand and market value of eri silk has attracted the other states also to practice ericulture where the food plant is available like West Bengal, Chhattisgarh, Andhra Pradesh, Uttar Pradesh, Gujarat, Madhya Pradesh, Tamil Nadu, Uttar Pradesh, Karnataka, Maharashtra, Sikkim, Jharkhand, Bihar, Orissa, and many others on a smaller scale (Rao, 2003).

Mulberry silk is produced extensively in the states of Karnataka, West Bengal and Jammu & Kashmir. Similarly, Tasar silkworms are reared traditionally by the tribes of Madhya Pradesh, Bihar, Orissa and Jharkhand. Muga and Eri silk are produced exclusively in Assam and adjoining region. Sericulture serves as an important tool for rural reconstruction, benefitting the weaker sections of the society, it provides not only periodical return within a short period of time but also assures own family employment opportunities around the year (Lakshamanan and Geethadevi, 2005)

Majority of the host plants of eri silkworms is available in the North-eastern region and the congeniality of the climate for eri silkworm has suited ericulture in the region (Vijayan et al., 2005). Teotia and Bajpeyi (2009) found that rearing of Eri silkworms is almost a household affair among the Bodo Community in Kokrajhar District. Sarmah et al. (2011) also reported that the tribal people consider Eri pupae as delicacy.

2.2 Evaluation of host plants

The distribution of moths and the number of individuals of any species in any locality, according to Scott (1933), is inextricably linked with the type of food plant available in that area. The nutritional value of food plants, either separately or in combination, is essential for the growth of larvae and the production of silk. The nutritional value of the leaf greatly influences the growth, development, and economic characteristics of silkworm (Krishnaswami et al., 1971).

Raychaudhury (1974) stated that the quality of leaf fed to silkworm influences the growth and development and silk production. The dietary efficiency of the food plants from which the silkworm obtains their nutritional requirements is determined by their chemical composition. Although most food plants contain all of the nutritional requirements, the quantity of each nutrient may not be well balanced for proper growth and development of silkworm. Quantifiable prerequisites for the required nutrient for optimum nutrition may vary within and between species due to a variety of factors such as the insect's synthetic ability and metabolic activities involving specific interconnections between certain nutrients (House, 1974). Silkworms feed in order to obtain and store sufficient amounts of energy, nutrients, and water from the food that they devour (Krishnaswami et al., 1978).

Vishwakarma and Thangavelu (1981) studied the influence of different season on larval weight and found that the larval duration varied by 3-4 days during different seasons. They also observed that the rearing performance was better when the silkworms were reared using castor leaves as compared to the silkworms reared using tapioca leaves. It was also noted that the larval weight has direct effect on the rearing performance like fecundity, effective rate of rearing, hatching percentage, survival rate.

Scriber and Slansky (1981) and Slansky and Scriber (1985), critically reviewed on the aspects of nutrition ecology of insect and concluded that nutritional indices as well as growth and development of insect varied on different host plants.

The eri silkworm is polyphagous, feeding on the leaves of numerous plants primarily from the families Euphorbiaceae, Araliaceae, Apocynaceae, and Simarubiaceae (Chowdhury, 1982). Chowdhury (1984) reported that the colour of the cocoon depends on the pigment absorbed from the leaves and these variation in cocoon colour is due to the permeability of cell wall and silk gland as a result of which the pigment come out along with excrement.

Thangavelu and Borah (1986) studied the effect of different host plants on eri silkworm and found that the cocoon quality was superior when the silkworms were reared using castor leaves, followed by Kesseru, Tapioca and Barkesseru.

The primary factor governing the production of good cocoon crops has been identified as the quality leaves offered to the worms for feeding. Superior-quality leaves increase the likelihood of good cocoon production in terms of quantity and quality (Ravikumar, 1988).

Pathak (1988) studied the influence of four different food plants like castor, tapioca, Gulancha and Kesseru on the nutrition, growth and cocoon characters of eri silkworm, *S. ricini*. He concluded that the food plants have significant role on the larval weight and duration of life cycle as well as the cocoon characters of silkworm. The higher larval weight and cocoon weight, shorter duration of larva and pupa, shell ratio was observed in the silkworms reared using castor leaves followed by Kesseru, Tapioca and Gulancha.

Kotikal et al. (1989) found that in females *S. ricini,* larval weight has a direct effect on the rearing performance. They observed that the pupal parameters such as weight and pupal size are direct indicator of fecundity.

Reddy et al. (1989) also reported the influence of host plants on the development and silk yield of eri silkworm while rearing using different host plants viz. castor, tapioca, ailanthus and plumeria. It was reported that the silkworms have shorter life cycle (46.49±1.32days), high survival rate (95.67±2.02%), higher shell ratio (12.2±0.17%) and higher reproductive rate when the silkworms were fed on castor leaves. Naik et al. (2010) also observed similar findings while comparing the rearing performance of *S. ricini* reared using castor and Indian almond leaves.

Raja and Samson (1991) studied on the influence of secondary host plant, *Cinnamon glanduliferum* on the growth parameters of eri silkworm, *S. ricini*. It was reported that the larval weight did not show significant variation as compared to the eri silkworms reared using castor leaves. Shell weight of the silkworms reared using *C. glanduliferum* was found to be higher (0.344 g) as compared to silkworms reared using castor (0.353 g) and Kesseru (0.32 g). However, the larval duration, fecundity, hatching percentage and effective rate of rearing was comparatively lower in the silkworms reared using *C. glanduliferum* leaves.

The growth, development, and even cocoon features can be affected by the plants that the silkworm eats. Moisture, total protein, total carbs, and total minerals are all metrics and elements of leaves of food plants that affect the economic aspects of silkworm harvests (Bongale and Chaluvachari, 1993).

Nagalakshmanna et al. (1988) also studied the influence of larval weight on fecundity and hatching percentage of *S. ricini* and reported that the weight of pupa has direct effect on the hatching percentage and fecundity.

Balamani et al. (1995) reported that the nutrient composition of mulberry leaves influences the growth of silkworm larva. It was also mentioned that micronutrients are equally important for the optimal growth of the silkworms along with the organic nutrients. The nutritional content of host plants acts as a major factor in the survival of non-mulberry silkworms (Pandey, 1995).

Hazarika and Hazarika (1996) evaluated the effect of castor varieties on the growth parameters of eri silkworm and reported the preference of castor variety. No significant variation was observed in the cocoon parameters however, the highest silk ratio was observed in the silkworms fed with red petiole variety as compared to the other ten varieties of castor leaves viz., red, local green, red petiole, powdery, RC-8, Aruna, GCH-4, GCH-2, CO-1 and TNV-5.

Seasonal fluctuations in a precise area, such as temperature, humidity, sunshine, rainfall governed by various geographical parameters, influences the growth and economic parameters of silkworm (Murthy et al., 1996; Clarke and Clem, 2003; Tamiru et al., 2012; Bhatia and Yousuf, 2014).

Khanikor and Dutta (1997) studied the effect of rearing eri silkworms on Borkesseru leaves and found that the rearing performance was found to be better on castor leaves during all seasons. They also suggested that Borkesseru can be utilised as food plant for rearing eri silkworm in absence of castor leaves. Selection of food plants with superior nutritional value is important for the healthy development of silkworms and in obtaining quality cocoon crops because the quality of the leaf has a direct impact on the health, growth, and survival of silkworms (Dutta et al., 1997).

Siddique et al. (1998) investigated the genetic variability and correlation of absolute silk yield and contributing traits in six eri silkworm ecotypes and discovered a wide range of phenotypic variability for absolute silk yield, fecundity, hatching, and effective rate of rearing. The range of variability in larval weight, cocoon weight, and shell weight was found to be comparatively lower. Larval weight provided the most accurate estimate of genetic covariance, followed by silk ratio and cocoon weight. Absolute silk yield was found to be significantly and positively related to fecundity, larval weight, cocoon weight, and shell weight.

According to Sannappa and Jayaramaiah (1999), mature larval weight and larval duration were associated with larval survival, effective rate of rearing, cocoon weight, pupal weight, shell weight, shell ratio, adult emergence, fecundity, and hatchability. They also found that increased larval duration and mature larval weight have an effect on rearing performance, as well as cocoon and grainage parameters.

Nangia et al. (2000) reported the host plant preference of *S. ricini* is in the order of castor, tapioca, papaya, barkesseru and gulancha on the basis of merit. Although castor and tapioca are the two most important host plants for eri silkworm, certain perennial tree species such as kesseru and payam in the north eastern states of India may provide supplementary nutrition during the off season.

Sujathamma and Dandin (2000) provided evidence that the mulberry silkworm was affected by differences in nutritional value between mulberry cultivars. Furthermore, the difference in the growth performance could be due the nutritional content of the leaves of different genotypes. Studies conducted by Jayaramiah and Sannappa (2000); Sannappa et al.

(2007); Sengupta et al. (2008) found differences in larval, cocoon and silk traits of eri silkworm when fed with the leaves of different genotypes of castor plant.

Sarmah et al. (2002) evaluated the effect of various genotypes of castor plants and reported that the performance of eri silkworm was found to be higher in the silkworms reared using red non-powdery variety followed by local green variety. Hazarika et al. (2003) mentioned that castor is best suitable in terms of various parameters like weight of larva, pupa, shell and cocoon, ERR, nutritive value, fecundity, hatching percentage, shorter larval and pupal duration. The survival, rate of food intake, digestion, and assimilation are all significantly impacted by the host plants, and these factors have a direct impact on the growth and development. Larval food intake affects a variety of parameters, including growth rate, larval duration, overall survival, and reproduction rate (Das and Das, 2003).

Castor (*R. communis*) is the most preferred host plant for eri silkworm followed by Kesseru (*Heteropanax fragrans*). The other available host plants include Tapioca (*Manihot esculenta*), Papaya (*Carica papaya*), Borpat (*Ailanthus grandis*), Borkesseru (*Ailanthus excelsa*), Jatropha (*Jatropha curcas*), Gamari (*Gmelina arborea*), Korha (*Sapiumeugenifolium*), Payam (*Evodia flaxinifloia*), Gulancha (*Plumeria acutifolia*) and many more (Singh and Das, 2006; Chakravorty and Neog, 2006; Bhattacharya et al., 2006; Das et al., 2006; Chowdhury, 2006; Bindroo et al., 2007).

Chakravorty and Neog (2006) evaluated the suitability of host's food plants like castor, tapioca, kesseru and payam and mentioned the superiority of castor in terms of fecundity, cocoon and larval weight, shell weight.

Chutia et al. (2009) carried out an extensive survey in Nagaland and collected 14 species belonging to 8 genera and recorded their food plants. They listed Castor (*R. communis*), Kesseru (*H. fragrans*), Payam (*E. fraxinifolia*) and Cassava/Tapioca (*M. esculenta*) for *S. ricini.*

According to Kumar and Elangovan (2010), food plant could be regarded as an indicator species. Effect of different host plants like castor, tapioca, jatropha and papaya on the volumetric attributes of eri silkworm was observed and it was found that ratio of silk gland volumes is directly linked to the size and weight of the larva. The maximum silk yield (14.74%)

and larval weight (7.38g), shortest larval duration (19.25 days), was observed when the larvae is fed with castor leaves which was followed by tapioca, papaya and jatropha.

The productivity and quality of cocoon, however, depends upon quality food supply, favourable environmental conditions and utmost hygienic condition (Yadav and Mahobiam, 2010). Castor is greatly exploited for eri silk production in non-traditional States of India whereas tapioca, the most preferred food plant after castor has also been proved to be suitable for commercial rearing (Sakthivel, 2012).

Shifa et al. (2014) studied the effect of castor genotypes on the rearing performance of eri silkworm and the result indicated that castor genotypes viz. Abaro, Acc 106548, Acc 203241, Acc 208624, Arsel, Bako, GK sel resulted in significant variation in the cocoon weight, shell weight, silk ratio, effective rate of rearing, fecundity, survival ratio of the silkworm.

Sharma et al. (2015) also evaluated the rearing performance of *S. ricini* reared using the combination of castor and different perennial food plants like kesseru, borkesseru and borpat and suggested that the perennial host plants could also be used for rearing of eri silkworm.

Das (2015) studied the effect of tapioca on rearing parameter of eri silkworm and suggested that tapioca can be used as a primary host plant for rearing *S. ricini* during the scarcity of castor plant. The cocoon parameters such as size, shape and colour also get influenced by the host plants used for rearing, the colour of the cocoon depends on the pigment absorbed from the leaves.

Ahmed et al. (2015) studied the rearing performance and reproductive biology of *S. ricini* feeding on *Ailanthus* species and other perennial food plants and revealed the superiority of the combination of castor with borpat leaves fed during early and later stages respectively which was at par with the economic parameters of the silkworm reared using borpat alone in terms of lower larval and pupal duration, high larval weight, cocoon and shell weight, effective rate of rearing (ERR). Rearing performance was found to be lower in the silkworms reared using the combination of castor and kesseru. In terms of fecundity, the silkworms reared using the combination of castor and borpat was recorded to be highest followed by the silkworms reared using the combination of castor and borkesseru.

Tungjitwitayakul and Tatun (2017) compared the effect of rearing *S. ricini* on artificial (Silkmate L4M) and natural diet (Cassava leaves) and found that the larval and pupal weight were higher in the silkworms reared using artificial diet however, the total haemocyte count, total haemolymph protein, lipid, and alpha-amylase activity were lower in silkworms reared using artificial diet as compared to the silkworms reared using cassava leaves. They concluded that the biochemical and biological parameters of the eri silkworms were influenced by the artificial diet used to feed the silkworms.

Birari et al. (2019b) evaluated the rearing performance of *S. ricini* on different host plants viz., castor, tapioca, adruso, banyan tree and Indian almond and revealed that the castor fed silkworms showed highest larval weight, larval survivability, ERR, cocoon weight, shell weight and shell ratio, emergence rate, fecundity, hatchability, male and female longevity and lowest larval duration, disease incidence, incubation period which was followed by the silkworms reared using tapioca and adruso.

Kamble and Jadhav (2019) conducted a study to estimate the morpho-economic traits of *S. ricini* by rearing on castor and papaya and suggested that castor leaves serves as chief host plant however, the combination of castor and papaya could also be used beneficially for commercial rearing of eri silkworm.

Thanga et al. (2021) conducted a study to identify the most suitable host plant for eri silkworm for sustainable silk production by rearing silkworms using different variety of castor and tapioca leaves and reported the superiority of castor variety YTP 1 in terms of economic traits including cocoon yield and silk percentage and concluded that high level of moisture content, protein and total carbohydrate content of the castor variety has led to the high rearing performance of eri silkworm.

2.3 Haemolymph composition

Insects have an open blood system, with blood filling the 'haemocoel' or the general body cavity. Insect blood, or haemolymph, is composed of liquid plasma and numerous blood cells, or haemocytes (Pandey and Tiwari, 2012), and it is a critical component of innate immune system. Haemocytes share many similarities with vertebrate white blood cells, allowing us to utilize haemocytes as a traceable model for immunoregulatory research and it plays a very crucial role in insect physiology as it is responsible for the coagulation of haemolymph (Gregoire, 1955). Role of haemocyte is also significant in defence mechanism of the immune system of insect against microbes, self and non-self-recognition, detoxification, cellular response, phagocytosis and encapsulation (Wigglesworth, 1959).

Zibae and Sendi (1959) also evaluated the haemoctyes of two lepidopterans, *Glyphodespyloalis* and *Hyphantriacunae* and described six types of haemocytes namely, prohaemocyte, plasmatocyte, granulocyte, oenocyte, spherulocyte and adipohaemocyte. Lauffer (1960) was the first to ever observed haemolymph proteins in silkworm *B. mori*.

Nittono (1960) classified the haemocytes in adult *B. mori* into six types namely prohaemocytes, plasmatocytes, granulocytes, spherulocytes and imaginal spherulocytes (observed only during adult stage but occasionally in the pupa on the day before emerging out of pupa) and oenocytes.

Akai and Sato (1973) reported that phagocytosis is one of the main functions of granular haemocytes in *B. mori* as they are the first cells to come in contact with foreign body in the initial capsule formation during phagocytosis which then release their granular content.

Haemolymph proteins are crucial for enzyme activities and transport in insects. Genetic and hormonal factors regulate the synthesis and utilisation of haemolymph proteins (Hurliman and Chen, 1974).

Haemolymph is the only extracellular fluid containing the products required for every physiological activity of the insect body. Thus, the changes in the composition of haemolymph reflect the physiological and biochemical transformations taking place in the insect tissues. The chemical composition of haemolymph is highly variable among species and at different developmental stage of the same species (Florkin and Jeuniaux, 1974). Gupta (1979) classified haemocytes into seven morphologically distinct types as prohaemocytes, plasmatocytes, granulocytes, spherulocytes, oenocytoids, coagulocytes, and adipohaemocytes. Plasmatocytes and granular cells are considered as the two main cells involved in all defence mechanisms in insects (Ratcliffe and Rowley, 1979).

The haemolymph of insects is the most important organ system where changes in biochemical constituents can be seen. Haemolymph serves as a reservoir for many materials required by a wide range of insects, and its composition changes in response to changing conditions or activities. The changes in structure, number and types of haemocytes influences the physiology and biological processes of the insect (Hirano and Yamashita, 1983; Adedokun and Denlinger, 1985). One of the major sources of haemolymph protein is the fat body. The haemolymph of insect performs a number of physiological tasks including immunity, transportation, and reserve storage (Mullins, 1985).

Bardoloi and Hazarika (1995) observed the influence of seasonal variation on body weight, lipid, blood and haemocyte content of *Antheraea assama* during spring, summer, autumn and winter and found that the silkworm showed higher level of blood and water content during spring and summer as compared to those reared during winter and autumn. Hazarika et al. (1994) reported that diet and food plants influence the population of haemocyte and blood in *A. assama.*

The immune response of various insect and caterpillar is dependent on the activities of two major haemocyte populations, granulocytes and plasmatocytes, which recognise pathogens and parasites and are immune system effector cells (Tojo et al., 2000).

The study on the defence function of haematocytes in insects revealed that the majority of capsules or nodules around masses of bacteria and necrotic melanized material *in vivo* are formed by plasmatocytes (Lavine and Strand, 2002).

Mallikarjuna et al. (2002) evaluated the influence of a systemic fungicide (Bayleton 25% WP) on total haemocyte count and biochemical changes in haemolymph of silkworm, *B. mori* infected with *Beauveria bassiana*. The total haemocyte count in the haemolymph treated with systemic fungicide increased for 5 days after inoculation, but in the inoculated control, it grew for just 3 days, showing a positive haemocyte mediated response.

According to Ling et al. (2003), florescence microscopy with different fluorochromes can distinguish between three types of circulating haemocytes in the silkworm, *B. mori*. It was discovered that on the basis of their fluorescence properties, the first category (granulocytes and spherulocytes) is positive for acridine orange and includes vivid green fluorescent granules.

Spherulocytes appear more homogenous and large and do not consist of any green fluorescent granules in the cytoplasm, whereas granulocytes' green granules are found to be heterogeneous and relatively small. Prohaemocytes were said to be round, and their dark nuclei were said to stand out clearly against the barely fluorescent green cytoplasm. Plasmatocytes were irregularly in shape and with invisible nuclei. After being stained with acridine orange, the prohaemocytes and plasmatocytes in the blood appeared very pale green. Oenocytoids along with their nuclei were reported to be positive for propidium iodide.

The two major haemocyte populations namely granulocytes and plasmatocytes accounts for roughly 90% of all haemocytes (Nardi, 2004; Levin et al., 2005).

Kerenhap et al. (2005) studied the influence of feeding frequency on the total and differential haemocyte count in eight bivoltine silkworms, *B. mori* breeds and revealed that starvation resulted in the decrease in total haemocyte count in these races of *B. mori*.

Almost all inorganic constituents such as electrolytes or ions, phosphates anions, organic constituents such as free amino acids, proteins, lipids, carbohydrate, and uric acids are found in haemolymph plasma. They are also responsible for transport of nutrients and proper growth of the insect species (Malik, 2009).

Haematological studies provide information about the physiological status of organisms as well as diagnostic keys for various changes and diseases. The haemolymph plasma contains almost all inorganic constituents like electrolytes or ions, phosphates anions, organic constituents like free amino acids, proteins, lipids, carbohydrate, uric acids (Malik and Malik, 2009).

Pandey and Tiwari (2012) presented an overview of insect haemocyte science and its future applications in applied and biomedical fields. In the study, they have generated a lot of information about various aspects of haemocyte science viz., different types of haemocytes, the haemocyte immune responses, various stresses (biotic as well as abiotic) affecting the population of haemocytes, key points and challenges and future applications of haemocyte science. In the review, effort has been made to correlate various responses of haemocytes against the change in order to propose haemocyte-based model in the form of catalogue to discern the health status and its future applications in applied and biomedical sciences.

The physiological state of the organism is indicated by haematological studies, which also offer diagnostic clues for various changes and illnesses. However, the present knowledge about haemocytes is limited to studies of not more than 200 insect species in about 200 genera mainly studies in Hymenoptera, Lepidoptera, Coleoptera and Diptera (Bhagawati and Mahanta, 2014).

Tungjitwitayakul and Tatun (2017) studied the effect of rearing *S. ricini* on cassava leaves and artificial diet and reported that the total haemocyte count, hemolymph protein concentration a total lipid concentration was higher in the larvae reared on cassava leaves while it was lower in the larvae fed with artificial diet.

Talukdar et al. (2015) studied the effect of lead on the haemogram of eri silkworms and highlighted the variation in the total hemocyte count of the silkworms reared using castor leaves as compared to the larvae reared on lead fortified castor leaves and mentioned the superiority of using castor leaves as the blood volume, total hemocyte count was higher in the silkworms reared using castor leaves.

Tungjitwitayakul and Tatun (2019) also studied the effect of rearing *S. ricini* on host plants (castor) versus on an artificial diet on different stages of silkworm. It was reported that the haemocytes of *S. ricini* are classified into five types: prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs), and oenocytoids (OEs) in which PLs were most abundant followed by GRs, SPs, PRs and OEs. The number of haemocytes in the silkworm reared on castor leaves was higher during early developmental stages and decreased gradually in later stages while the hemocyte count of the silkworm reared using artificial diet was lower in all stages. It was also mentioned that the diet had different effects on the percentage of PLs, GRs, and PRs during larval and pupal stages.

Similar studies by Gogoi et al. (2022) on the differential and total haemocyte count in fifth instar larvae of *S. ricini* reared on castor and tapioca leaves also revealed that five types of haemocytes are present in the hemolymph of *S. ricini* namely prohaemocytes, plasmatocytes, granulocytes, spherulocytes, and oenocytoids. It was also mentioned that the haemocytes of *S. ricini* reared on castor leaves has higher haemocytes count as compared to the silkworms reared on tapioca leaves.

2.4 Biochemical and nutritional analysis

Insects are proven to be an important source of food, especially in developing nations (Bodenheimer, 1951). Carbohydrates are the main components of diet of all living organisms which are directly or indirectly used as a source of energy for all essential activities. Wyatt and Kalf (1956) revealed that trehalose is the major component of carbohydrate in blood of insects. Amino acids, the building blocks of protein is an essential component of living cells. Many insects, including the silkworm, are known to contain a high concentration of free amino acids (Ramsay, 1958).

Proteins, carbohydrates, and lipids are major biomolecules that play an important role in the biochemical processes that supports growth and development of the silkworm (Ito and Horie, 1959).

High potassium and low sodium levels is typical characteristic of mineral content of plant foliage which is found in the haemolymph of phytophagous insects (Florkin and Jeuniaux, 1974). Amino acids are required for silkworm growth and survival. According to Shimura (1978), haemolymph acts as an amino acid reservoir between the midgut and the silk gland, supplying amino acids to the silk gland for silk synthesis.

The total concentration and the variation of protein of the haemolymph and silk gland are very significant in order to study their involvement in the ontogenetic growth and development of the insect and silk production. Sumioka et al. (1982) established the nutritional importance of proteins in silkworm development. Proteins are essential for growth and development, as well as the synthesis of silk proteins in the silk gland during larval development (Seo et al., 1985). Insect protein concentrations have been compared favourably to casein (Ozimek et al., 1985).

Distinct metabolic changes have been seen during the metamorphosis of eri pupae at varying temperatures (El-Shaaraway et al., 1982). Negative environmental circumstances trigger diapause preparation in insects. Insects enter diapause once their endocrine glands have been stimulated. Widely distributed insects may anticipate natural disasters, and thus they begin preparing for diapause from the larval stage and continue it during the pupal stage (Adedokun and Denlinger, 1985; Tauber et al., 1986; Leather et al., 1993). It is widely acknowledged that the neutral lipid found in silkworm pupae is an excellent source of alpha-linolenic acid (ALA). Even though there were variations in the level of ALA (traces to 40% of total fatty acids) reported so far in the *B. mori* silkworm pupae, based on numerous literature reports on the fatty acid composition of neutral lipids predominantly of pupae of *B. mori* (Werner, 1936 and Rao, 1994).

According to Simex and Kodrik (1986), the glycogen content of the fat body, body wall, and silk gland, as well as the free carbohydrates in the haemolymph, altered dramatically during the final larval instar and metamorphism in silk worms.

Silkworm pupae are a by-product of the silk industry that can be used as fertiliser, or included into animal diets like those for chickens and fish (Ichhponani and Malik, 1971; Rao, 1994). The silkworm pupae and oil are said to be rich in phosphorous and alpha linolenic acid (ALA), respectively. Eri pupal oil has the potential to be a source of ALA because it has been shown to be safe and nutritionally equal to typical vegetable oils (Longvah et al., 2012).

On a dry weight basis, insects have very high crude protein content, and it has been claimed that insect proteins are an excellent source of essential amino acids, comparable to or even superior than soy protein (Finke et al., 1989).

Protein, fat, carbs, and vitamins are all present in quite high concentrations in dry-weight terms in eri silkworm pupae. In addition to the pharmacological and therapeutic significance of pupa oil, deoiled pupae are a rich source of certain vital amino acids. Both pisciculture (Majhi et al., 1991) and protein extraction (Datta et al., 1993) make use of eri pupae. According to reports (DeFoliart, 1991; Chen and Feng, 1999; Feng et al., 2000), the majority of edible insects possess more fat and fatty acids throughout their larval and pupal stages than during their adult stage. According to Lu et al. (1992), highly varied fatty acid composition was observed among the edible insect species.

A high level of haemolymph proteins content may be associated to a higher consumption of mulberry leaves, resulting in a faster rate of conversion and storage in haemolymph (Banno et al., 1993; Aruga, 1994).

The pre-pupal or pupal stage of the eri silkworm is consumed by various North-East Indian ethnic groups as a delicacy and a staple food. These groups include the Rabhas, Bodos, Abor, Miri, Kachari, Garos, Khasi, Naga, Adi, and Mizo (Roychoudhury and Joshi, 1995).

Muniandy et al. (2001) reviewed the literature on mineral availability in plants and insects. Zinc is considered to have a role in preventing apoptosis and oxidative stress, among other cellular activities including catalysis and gene expression. The cofactor roles of iron and zinc are thought to be fair universal importance to organisms, therefore it is reasonable to believe that trace elements like these are necessary for insects in general (Nichol et al., 2002).

Some fatty acids are called "essential" because the human body needs them but cannot produce them on its own. Omega-6 fatty acid shortage can cause poor development, fatty liver, skin lesions, and even reproductive failure; it is one of the two main families of polyunsaturated fatty acids (PUFAs), along with omega-3 fatty acid. It has been shown that the essential fatty acid omega-3, commonly known as alpha-linolenic acid, can help reduce the risk of developing coronary heart disease (Ascherio, 2002). Increased consumption of linoleic acid has been linked to lower blood pressure, reduced aggregation of platelets, and improved erythrocyte deformability (Iso et al., 2002).

The eri silkworm is one of several insects consumed by the locals of north-east India. The prepupae and pupae of the eri silkworm are considered to be delectable in northeastern India. The consumption is not only limited to eri silkworm as other species, including muga (*A. assama)* and mulberry (*B. mori*) are also consumed. However, the consumption was highest for eri (87.7%), followed by muga (57.4%) and mulberry (24.6%) (Mishra et al., 2003).Rao (1994) and Mishra et al. (2003) also mentioned that overall consumption was highest for *S. ricini* (87.7%) followed by *A. assama* (57.4%) and *B. mori* (24.6%). Proximate analysis suggested the pupae of silkworm as a good source of protein and fat.

The desilked pupae that are produced as a by-product of ericulture are prized for their delicious flavour and excellent nutritional content (Singh and Suryanarayanan, 2003). This is because they contain a high percentage of both protein and fat. Nevertheless, the ingestion of silkworm pupae after they have completed their life cycle is not common among the general population.

Fatty acids are the building blocks of fat. Diglycerides and triglycerides are formed when two or three fatty acids are coupled to glycerol respectively. Based on their degree of saturation, these fatty acids are classified as saturated, mono-unsaturated, and poly-unsaturated. Polyunsaturated fatty acids are classified as omega 3, 6, or 9 fatty acids based on the position of the first double bond. The fat content of insects varies between 10 and 70% on a dry matter basis (Bukkens, 1997; Finke, 2004; Yang et al., 2006).

Insect protein is often lacking in sulphur-containing amino acids but rich in lysine and threonine, two essential amino acids that may be lacking in grains and cereals. Insects are also a great source of the healthy fats besides the vitamins and minerals they provide (Oliveira et al., 1976; Malaisse and Parent, 1980; Banjo et al., 2006).

Zhou and Han (2006) evaluated the chemical composition and nutritional quality of *A. pernyi* and found that the pupal powder contained 6.6 % water, 71.9 % crude protein, 20.1 % fat, and 4.0 % ash (dry matter). The mineral analysis showed low Na/K ratio and low heavy metal concentration in addition to a high K content. All of the eighteen known amino acids were present in the pupal protein; this included all of the necessary amino acids and the sulphur-containing ones. Pupal protein was of good quality since it included all nine necessary amino acids, as specified by Food and Agriculture Organization of the United Nations/ World Health Organization (FAO/WHO).

Haemolymph is the primary circulating fluid and a flowing repository of roughly about 241-298 proteins (Lix et al., 2006) that support larval development, metamorphosis, silk synthesis, apoptosis, chitin and haemocyte creation, salivary gland and reproductive organ development, and ecdysis (NagaJyothi et al., 2010).

Insects serve as a significant source of critical amino acids and contribute 10–30% of essential amino acids, such as phenylalanine, lysine, threonine, tryptophan, and tyrosine, to the human diet, according to World Health Organisation reports (2007).

According to a number of studies, certain edible insects with high polysaccharide content may improve immunity and contribute to human nutrition (Long et al., 2007). According to Hazarika (2008), the tribal populations of Assam, Manipur, and Nagaland love to eat the prepupae and pupae of eri silkworms (*S. ricini*), muga silkworms (*A. assamensis*), and mulberry silkworms (*B. mori*) as a delicacy.

The nitrogen and crude protein content of foliage, as well as free amino acids were found to have a substantial impact on the weight of larvae and cocoons (Vijayakumar et al., 2009). The body of an insect may include fats in a variety of ways. According to reports (Ekpo et al., 2009), triacylglycerols make up more than 80% of the fat in insect body fat. Each edible insect species has a very different level of carbohydrate content. In certain commercially available edible insects, Raksakantong et al. (2010) found that the carbohydrate content ranged from 1 to 10% of the dry matter.

Mishra et al., (2010) studied the changes in the biochemical constituents of the haemolymph of trivoltine eco race of *A. mylitta* and found that while the glycogen, trehalose content was found higher in females however, higher level of amino acids and proteins was observed in male larvae. They also concluded that the fourth instar larvae of trivoltine variety of tasar silkworm accumulate energy and store it for their use during the pupal stage. Anjani et al. (2010) discovered a substantial positive relationship between total phenol content and insect resistance. Phenols are known to play a vital role in plant defence against harmful insects, and this has been demonstrated in numerous crops.

According to reports, desilked silkworm pupae have a high nutritional value due to their high protein and fat content; these are used as a dietary supplement (Wang et al., 2010). Longvah et al. (2011) studied the nutrient composition of prepupae and pupae of *S. ricini* and concluded that the silkworm, *S. ricini* is a good source of fat, protein and minerals. The amino acid profile has revealed that it is a good source of essential amino acids like histidine, isoleucine, leucine, threonine, valine etc. with a high PDCAAS score. They also suggested that the defatted eri pupae could be used as feed for animal nutrition due to the presence of high protein which can also generate further income from waste product of the silk industry.

Over two billion individuals throughout the world already use edible insects as a beneficial source of food, owing to their accessibility and nutritional value (Premalatha et al., 2011). Sarmah et al. (2011) investigated the biochemical properties and rearing performance of the eri silkworm and discovered that larval weights were favourably connected with lipid and sugar contents and negatively with leaf nitrogen and protein contents.

Velide (2012) studied the effect of cold stress on the biochemical components of the larval haemolymph, fat body and silk gland of tasar silkworm, *A. mylitta* and revealed that exposure to low temperature (10 ºC) for seven, five and two days caused 100%, 54% and 22% mortality respectively when returned to normal temperature. The protein content was increased during low temperature while there was a decrease in fat body, silk gland and carbohydrate content. According to research by VanHuis et al. (2013), tropical and humid regions have the highest diversity of edible insect species.

Devi and Yellamma (2013) studied the protein profiles in *B. mori* fed with mulberry leaves fortified with trace elements such as zinc, vitamin, pyridoxine and methoprene hormone in different tissues like silk gland, haemolymph, fat body and muscle. It was found that among all the tissues, fat body has the highest content of total protein and haemolymph contains the least total protein. The total protein content was higher in the silkworm fed with the mixed dose of the trace elements as compared to the mulberry fed silkworms. The synthesis of new muscle tissue and other tissues is dependent on the metabolism of proteins. The silkworm's nutritional needs are not adequately met by a single protein source. Qualitative and quantitative changes in the amino acid content of proteins appear to reflect the variances in nutritional value. Protein levels are triggered by zinc ions entering cells in the silk gland, haemolymph, fat body, and muscle. Variations in silkworm body size can be attributed to fluctuations in protein levels. Vitamins and minerals have been shown to stimulate insect feeding.

Proteins are essential for development, metamorphosis, and the maintenance of many physiological functions (Kumar et al., 2011; Murthy et al., 2014). According to Rumpold and Schlüter (2013) and Kouřimská and Adámková (2016), some insects with high levels of lysine, threonine, and tryptophan had more nutritional value than some cereal proteins.

Lepidoptera caterpillars have a relatively higher fat content than other edible bug species. Total fat content was found to vary between 8.6g and 15.2g/100g in caterpillars, whereas it was slightly lower in grasshoppers and other Orthopterans (Tzompa-Sosa, 2014).

In multivoltine (Pure Mysore PM), crossbreed (PM×CSR2), and bivoltine (CSR2) silkworms, total body proteins rapidly increase from the first instar to maximum at the end of the fourth instar larval stage (Murthy et al., 2014).

Paul and Dey (2014) also assessed and compared the composition of lipids, amino acids, and fat-soluble vitamins A and E in frozen and oven-dried samples of *B. mori* to that of common food products. Stearic acid was discovered to be present solely in frozen form among the saturated fatty acids (SFAs). However, among the monounsaturated fatty acids, only oleic acid was found in dried sample. The frozen form of silkworm sample found to contain omega-6 fatty acids such as linolenic acid, g-linolenic acid, dihomo-g-linolenic acid, and arachidonic acid, as well as fatty acids such as a-linolenic acid and docosahexaenoic acid, indicating its potential to support cardiovascular function, whereas the oven-dried form contained linolenic acid, eicosatrienoic acid, eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA).

According to research, people from 300 different ethnic groups in 113 different countries worldwide consume more than 2000 different types of edible insects as food (Jongema, 2015). According to Kouřimská and Adámkovás (2016), the fat content of the majority of edible insects ranges from 10 to 60% by dry weight. Triacylglycerols are said to be used by insects as a source of energy during times of high energy demand, such as diapause, pupation, and extended flights, among other things.

Ravinder et al. (2016) studied the total lipid content and recorded the presence of 18-20% lipids and the isolated phospholipids of eri-pupal oil contain 96.72-97.33% of neutral lipids, 0.51-0.21% of glycolipids and 2.65-2.39% phospholipids. The lipids of the larvae fed with castor leaves showed higher content of neutral lipids than tapioca fed pupal lipids, however, polar lipids were higher in tapioca fed pupal lipids. They also stated that Alpha linoleic acid (ALA) was the major fatty acid followed by palmitic and oleic acid in tapioca fed lipids as compared to castor fed lipids. The lipid content of the larval oil varied due to the influence of host plants.

Transfer of biochemical constituents from different tissues may be necessary to satisfy the greater physiological activity in silkworm such as enhanced body development and cocoon creation. Hence, biochemical parameters like proteins, amino acids, carbohydrates, lipids, nucleic acids etc., alter dramatically during the life cycle of all living creatures (Ponmurugan and Karthikeyan, 2017).

Similar to other widely used biomedical textiles like polyester, silk has a number of polar functional groups that could optimise the absorption of antibiotics. Silk is a biodegradable, environmentally friendly polymer with superior moisture-absorbing and de-absorbing capabilities. The two proteins sericin and fibroin work together to form the silk fibre, which is created from soluble protein found in the haemolymph. Many biochemical components found in haemolymph are used as starting points for the synthesis of sericin and fibroin. Recently, research on sericin and fibroin in the area of drug delivery systems has been conducted (Sharma and Kalita, 2017).

Similar study was done by Mazumdar (2019) on the biochemical and nutritional composition of eri pupae. The crude protein in eri pupae was found to be 59.40%, 54.25% soluble protein, 5.85% crude fibre and the eri pupae is also found to contain essential minerals like calcium, phosphorous and iron. The good quality of the lipids in eri pupae is shown by their specific gravity, refractive index, iodine value, saponification value, and acid value, as well as the presence of cholesterol, neutral lipid, glycolipid, and phospholipid.

The amino acids are generally grouped as essential and non-essential amino acids while both are required, non-essential amino acids cannot be synthesized by most of the animals therefore, it should be provided through diet (Oonincx and Finke, 2020).

The quantification of vitamins has not been mentioned before this instance, little is known about the efficiency with which insects use minerals and trace elements. Although minerals and vitamins have a deleterious effect at greater doses on the proteins in the haemolymph, they have a considerable favourable effect at lower doses. In addition to these roles, the haemolymph also acts as a temporary storage organ for a wide variety of proteins and enzymes until they are absorbed and used by other organs, particularly the silk gland. Zinc may act as a modulator of proteins in the haemolymph's circulating medium (Liu et al. 2022).

2.5 Antioxidant scavenging capacity and immunological response of *S. ricini*

According to multiple investigations, the high-quality protein found in silkworm pupae may have diverse medicinal effects in the human body (VanHuis et al., 2013).

Gaíva et al. (2003) found that silkworm pupae oil has a noteworthy impact on prostaglandins, which helps in the prevention of prostate disorders. Additionally, it enhances the functioning of beta cells in diabetes patients by restoring the activity of fatty acid desaturase in cells, leading to increased insulin secretion.

Lemaitre and Hoffmann (2007) studied the immune defence mechanism in *B. mori* and reported that the primary defence of the insect body prevents infection by structural barrier and the secondary defence mechanism is provided by hemolymph through cellular and humoral response. Thus, insects have powerful defence mechanisms to fight infections by possessing an efficient innate immune system.

Yesmin et al. (2008) reported that the reducing power of a compound is related to its electron transfer ability which can serves as a significant indicator of its antioxidant activity. Additionally, the phenolic compounds were directly correlated with its antioxidant ability. The physiological effects of flavonoids include possible antioxidant activity.

Butkhup et al. (2012) also investigated the phenolic content and antioxidant activities of silk sericin extract of *B. mori* and *S. ricini*. The study revealed the presence of flavonoid compounds like catechin, quercetin and epicatechin in the silk sericin extract. They also highlighted that the Silk sericin extract of *B. mori* and *S. ricini* has high antioxidant activities and that the total phenolic and flavonoid content of silk sericin extract is positively correlated to the scavenging activity of 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) (ABTS) and Ferric ion reducing antioxidant power assay (FRAP). Shantibala et al. (2014) also reported that the silkworm, *B. mori* and *S. ricini* possess good antioxidant property.

Deori et al. (2014) documented the remarkable antioxidant and antigenotoxic properties of pupae from the muga silkworm, *A. assamensis*. It was reported that the scavenging activity of methanolic pupal extract and ascorbic acid showed IC_{50}values of 25.83 and 27.21 mg/mL and percentage inhibition of 73.33 and 91.05% at a concentration of 80 mg/mL respectively which makes it a good source of antioxidant and recommended the frequent consumption of the pupa of *A. assamensis* since the pupae have a great antioxidant potential to scavenge free radicals and a good antityrosinase activity

Ramappa et al. (2017) studied the antioxidant properties of oils extracted from the pupae of 4 silkworm species, namely *A. mylitta* (tasar silkworm), *A. assamensis* (muga silkworm), *S. ricini* (eri silkworm), and *B. mori* (mulberry silkworm). They found out that the pupal oil extract of silkworm contains α-Linoleic acid and it also showed superoxide radical scavenging activity that increases in dose-dependent manner. They also suggested that pupal oils can be used as a complementary food with good antioxidant potential.

Chukiatsiri and Hangtrakul (2018) studied the biological activities of protein extract of pupae *B. mori* and *S. ricini* and reported that the pupal extract of *B. mori* and *S. ricini* showed good antioxidant property for DPPH, ferrous ion chelating activity. The pupal extract also showed albumin degradation, α-amylase and acetylcholinesterase inhibition activity. Additionally, the pupae of *S. ricini* exhibited higher anti-diabetic activity by inhibiting α-glucosidase and iron chelating activity. They also reported that the protein extracts from silkworm pupae had biological activities against most of the causes of Non-communicable Diseases which may offer a possibility of prevention and treatment in the future.

Mattia et al. (2019) examined the antioxidant qualities of several commercially significant edible insects. They found that silkworms, grasshoppers, and crickets exhibited much stronger antioxidant properties compared to fresh orange juice. Lokeshwari et al. (2019) reported the DPPH radical scavenging activity of methanolic extract of *S. ricini, B. mori* and *A. proylei.* It was reported that the methanolic extract of *S. ricini* contains higher antioxidant activity among the three species of silkworm.

Ghosh et al. (2020) examined the antioxidant characteristics of reeling waste pupae from mulberry (*B. mori*), tasar (*A. mylitta*), and muga (*A. assamensis*) silkworms using different antioxidant assays such as DPPH, ABTS, superoxide, and reducing power. They found that the methanolic extract of *B. mori* contains stronger DPPH scavenging activity (IC_{50} of 28.40 μg/mL) as compared to *A. assamensis* (IC_{50} of 29.31 μg/mL) and *A. mylitta* (IC_{50} of 40.74 μg/mL). DPPH scavenging activity of the pupae also correlated significantly with ABTS radical scavenging activity and reducing power assay. The study also suggested that reeling waste pupae could be used effectively as natural antioxidant in the development of protein rich food products for use in animal feed sectors.

Variation in immune defence caused by nutritional contents of the host plants were studied in different insects such as the cabbage looper, *Trichoplusia ni* (Shikano et al., 2010), the European grape berry moth, *Eupoecilia ambiguella* (Vogelweith et al., 2015) and

Grasshopper, *Schistocerca gregaria* (Emad et al., 2016) and *Spodoptera litura* (Vengateswari et al., 2020). They reported that the variety of host plants used for feeding the insects brings changes in the haemolymph biochemical composition which in turn reflects the biochemical modification in immune response of the insect body.

Mahmoud (2020) also studied the humoral immune response of *B. mori* fed on different mulberry varieties. It was highlighted that the defence mechanism in *B. mori* is provided by open circulatory system through humoral and cellular responses. Results indicated that the type of mulberry varieties used and bacterial infection had significant effects on enzymes activity in the haemolymph of the larva. It was also reported that silkworms reared using *Morus alba* and *Morus laevigata* was superior in the providing humoral immune response.

From the available literature and information available from the previous studies done on eri silkworm, it is evident that eri silkworm, *S. ricini* is one of the most exploited silkworms in terms of silk production as well as for the production of pupa and pre-pupa which is considered delicacies and consumed in the North-eastern part of India. Ericulture is a household practice in the Seri-villages of Kokrajhar district and it is one of the important bio resources of North-east India. It is already known that the food plants of the silkworm contribute to the haemolymph of the silkworm and therefore, affect the total and differential count. The selection of food plants is also crucial in terms of growth, development and cocoon quality of the silkworm. The host plants also affects the biochemical and nutritional content of the silkworm. The immunity of the silkworm is also affected by the type of host plants used for rearing. The majority of the eri farmers use castor leaves for rearing however the annual nature as well as the high cost of plantation of the castor plant affects the eri production, therefore, it is necessary to find a suitable alternative to castor which can boost the silk production throughout the year .

The literature available on the nutritional content of *S. ricini* is scanty and much work has not been done on the scavenging activity and immunological responses of silkworm *S. ricini* fed on the different food plants. Therefore, because of all the above review this study has been proposed with the following objectives.

1. To study the total and differential haemocyte count in hemolymph of eri silkworm, *S. ricini* reared on different food plants.
2. To compare biological and biochemical parameters of eri silkworm, *S. ricini* reared on different food plants.
3. To study nutrient composition, scavenging as well as antioxidant activity and immunological capacity of eri silkworm, *S. ricini* reared on different food plants.