CHAPTER 5
DISCUSSIONS

The result of the investigation on the study of biochemical and immunological responses of Eri silkworms, *S. ricini* fed on different host plants have been discussed in this chapter.

5.1 Total and Differential Haemocyte count

5.1.1 Total Haemocyte count

The total haemocyte count (THC) was found to be highest in the silkworms reared using *R. communis* leaves (Sample C) followed by the silkworms reared in *M. esculenta* leaves (Sample T). According to Paul et al. (1992), the larval feeding efficiency increases haemocyte count in insects. However, the study done by Bardoloi and Hazarika (1995); Ambrose et al. (1999); Ling et al. (2005) showed the decrease in the total number of haemocytes during the developmental stage in other insects such as *Ephestia kuehniella*, *A. assamensis*, and *Rynocoris marginatus*. It was reported by Yang et al. (2008) that the THC varies with the developmental stage of the insect as well as the nutritional and physiological condition of the silkworm. They also found that the overall haemocyte counts of the silkworms reared on *R. communis* leaves was significantly higher than the rest which suggest that the diet of larvae influenced the number of haemocyte counts.

According to Lavine and Strand (2002), insects lack an acquired immune system but possess an established innate immune system. Haemocytes are blood cells in invertebrates, including insects, that play a crucial role in various physiological functions, mainly immune response, nutrient transport, clotting, defence against pathogens and are responsible for recognizing, attacking, and eliminating foreign threats. The haemocyte is the primary component of the innate immune system of the insects that directly eliminates pathogens from the body (League and Hillyer, 2016). Vogelweith et al. (2016) reported that the larvae reared using *R. communis* leaves have better physiological content which enabled them to produce a greater number of haemocytes as compared to the silkworms reared using different host plants.

According to Tungjitwitayakul and Tatun (2017) different diets such as artificial diet, Silkmate L4M influences and affects the duration of larval, pupal period, THC, Differential haemocyte count (DHC) and other biochemical parameters of the silkworm. Based on the THC and DHC of silkworm reared on *R. communis* leaves and artificial diet, the higher THC was observed during the larval period and decreased during the late larval and pupal period which remained persistent throughout the late larval and pupal stage (Tungjitwitayakul and Tatun, 2019).

Similar observation was recorded by Gogoi et al. (2022) where the total THC differed among the silkworms of the same species based on the type of host plants used for rearing. They also noted that the silkworms reared on *R. communis* and *M. esculenta* showed significant difference in the morphology and haemocyte count due to the different nutritional content of the plants used for their rearing.

5.1.2 Differential Haemocyte count

Gupta and Han (1988) identified and classified the insect haemocytes based on morphology and functions by using histochemical techniques. Various studies suggest that haemolymph of eri silkworm contains five types of haemocytes viz., Prohaemocytes, Plasmatocytes, Oenocytes, Granulocytes and Spherulocytes. The hemocyte in insect has diverse functions including phagocytosis, a process by which the haemocytes engulf foreign particles and prevents infection, and encapsulation, a major immune response where the haemocytes surround the foreign bodies and encapsule them. The haemocytes are generally classified based on the morphological characters and functions (Lavine and Strand, 2002; Ribeiro and Brehelin, 2006; Strand, 2008).

In the present study, the Plasmatocytes (PL) and Granulocytes (GRs) were found to be the most abundant type of haemocytes among the five haemocytes studied. However, the number of PLs and GRs varied among the silkworms reared using different host plants. The silkworm rearing using *R. communis* leaves (Sample C) was found to contain highest number of Plasmatocyes (PL) and Granulocytes (GRs), followed by the silkworms reared using *M. esculenta* (Sample T) and *H. fragrans* (Sample K) while the lowest number of PLs and GRs was found in the silkworms reared using *C. papaya* leaves (Sample P). The OEs are present in the least numbers as compared to the other types of haemocytes in the larvae of silkworms reared with different host plants.

According to Shapiro (1979) and Kurihara et al. (1992), the OEs are the least abundant hemocytes and they are likely to be absent during the early larval stages. They also found small proportion of OEs in larvae of many insects such as *G. mellonella, S. litura, D. saccharalis and L. oleracea*.

Takahashi and Enomoto (1995) also found that the haemocyte of fifth instar larvae of *S. ricini* had abundance of PLs and GRs as compared to PRs and OEs. The higher percentage of PLs and GRs was likely because of the reason that it can differentiate into Spherulocytes and Oenocytes (Han et al., 1998). Mahalingam and Muralirangan, (1998) also stated that the relative abundance of different types of haemocytes is not constant which is believed to be related to the type of host plant used for rearing and related to the molting activity of the insects. The study by Tungjitwitayakul and Tatun, (2019) also suggest that larval diet (artificial) affected the abundance of five haemocytes during third to fifth instar resulting in increase of PLs and decrease of SPs during fifth instar. The larvae in fifth instar are extreme feeders therefore, they are sensitive to changes in nutrition and composition of the diet.

As per Yamashita and Iwaabuchi (2001), spherulocytes helps in synthesis and secretion of silk protein while Prohaemocytes are the stem cells that gives rise to other haemocytes and are responsible for continuous replenishment of haemocytic population. OEs are found to be involved in the production of prophenoloxidase, an enzyme involved in humoral immunity and apoptosis (Lavine and Strand, 2002). According to Ling and Yu (2006), PLs and GRs are found to have a crucial role for the cellular immune response of the silkworms and provide wide range of immunity.

Thus, the total haemocyte counts in *S. ricini* serves as an important parameter in assessing the overall health and immune response as well as in maintaining resistance against pathogens. In addition, total haemocyte count also acts as an indicator of general immune status of the silkworm as it is the reflection of the total number of haemocytes circulating in the body. The influence of the host plant was seen in growth and development of the silkworm. The total number of haemocytes may increase in response to infection or external factors like stress, nutrition, etc. According to Schmitz et al. (2012), the granulocyte haemocyte found in Eri silkworm is responsible for engulfing and digesting foreign particles, hence, serving as primary defence against the pathogens. Another important haemocyte, the plasmatocytes is crucial for wound healing and clotting mechanism. OEs are associated with encapsulation and SPs are responsible for coagulation, storage of energy and transport of lipids. Recently Gogoi et al. (2023) also found that the haemocyte of fifth instar larvae of *S. ricini* abundant on PLs and GRs as compared to PRs and OEs.

5.2 Study on the economic parameters of *S. ricini* fed on different host plants

The present study was done to study the economic parameters of *S. ricini* based on the hosts plants selected for rearing i.e., *R. communis* (Sample C), *M. esculenta* (Sample T), *G. arborea* (Sample G), *H. fragrans* (Sample K) and *C. papaya* (Sample P). This study observed variations in larval duration among groups of silkworms reared on different host plants across different seasons. During summer (S2), the silkworms was observed to have the shortest larval period. Among these, *R. communis* fed silkworms (Sample C) had the shortest larval period, followed by *H. fragrans* (Sample K) and *M. esculenta* (Sample T) fed silkworms, while *C. papaya* (Sample P) fed silkworm had longest larval period. Summer showed shorter periods compared to winter, likely due to nutritional differences in the leaves and the impact of high temperatures accelerating larval development, while lower temperature during winter prolonged larval duration.

Regarding pupal period, *R. communis* fed silkworm (Sample C) exhibited shortest duration, whereas those fed on *C. papaya* leaves (Sample P) had the longest pupal duration. The study suggested the pupal duration for eri silkworms ranging from 11 to 17 days depending on the type of host plant used for rearing. According to Sannappa et al. (2007) the choice of host plant could impact the pupal duration resulting in longer pupal period in the larvae fed with certain castor genotypes.

The observation was at par with the findings of Subramanianan et al. (2013) and Shifa et al. (2015) who reported that the larval period of eri silkworm could range from 22.67 to 25.83 days. Kumar et al. (2018) also reported that the larvae of eri silkworm reared using *R. communis* leaves had a larval period of 22 days whereas, the larvae fed with additives had an influence on the larval period resulting in a shorter or longer larval duration. On the other side, Sarmah et al. (2012) identified autumn as the most productive season for eri silkworm rearing. The difference may be due to difference in the climatic condition and the quality of host plants used for rearing.

Similarly, Ahmed et al. (2015) also reported from their research on the rearing performance of eri silkworm feeding on *Ailanthus* and other promising food plants that the larval period of eri silkworm was lowest (18.33 days) during July-August when using *R. communis* (for first and second instar) and Borpat (*Ailanthus grandis*) (for third to fifth instar). Conversely, silkworms reared on *H. fragrans* leaves (Sample K) during winter exhibited longest larval duration. However, Sharma and Kalita (2017) suggested that seasonal variation have profound effect on pupal duration. The findings were corroborative with the study by Birari et al. (2019b) who reported that the pupal duration of *S. ricini* was found to be 15.73 days. Studies by Borah et al. (2021) also reported shorted pupal period during summer season.

Fecundity was found to be highest in the silkworm reared using *R. communis* (Sample C) which was comparable to the fecundity of the silkworms reared using *M. esculenta* (Sample T) and *H. fragrans* (Sample K). The lowest fecundity was observed in the silkworms reared using *C. papaya* leaves (Sample P). The fecundity was also observed to be influenced by the types of host plants used for rearing. Sarkar et al. (2015) reported variation in the fecundity of eri silkworms reared using different castor genotypes. Ahmed et al. (2015) also recorded highest fecundity in silkworms reared using *R. communis* combination with *Ailanthus grandis* (Borpat). The average fecundity observed in the present study was similar to that observed by Birari et al. (2019b) who reported that a female eri silkworm lays an average of 360.1 eggs. However, Saratchandra and Joshi (1984) and Ravishankar et al., (2000) reported variations in fecundity.

Different host plants and season have been found to impact the hatching percentage of eri silkworm significantly. The influence of host plants and seasonal variations were seen in the hatching percentage of *S. ricini*. The hatching (%) was found to be higher during Season 1 (S1) for silkworms reared using *R. communis* (Sample C), *M. esculenta* (Sample T), *G. arborea*(Sample G) and *C. papaya* (Sample P) however, in silkworms reared using *H. fragrans* (Sample K), hatching (%) was highest during Season 2 (S2) which was supported by the studies of Kumar and Elangovan (2011) who observed 93 to 95% hatching percentage during spring, 2007 when the temperature was 22-26°C and 93.75 to 95.20% during spring 2008 when the temperature was 23-28°C. It was also reported that the fluctuation in temperature and humidity during different seasons has a direct impact on the hatching percentage of the silkworm.

The effective rate of rearing (ERR) is intricately related to the kind of host plant used to rear the eri silkworms and the seasonal variations. The ERR (%) was found to be highest during S1 for the silkworms reared using *R. communis* (Sample C)*, M. esculenta* (Sample T), and *G. arborea* (Sample G) while the ERR (%) of the silkworms reared using *H. fragrans* (Sample K)*,* was higher during S2. The lowest ERR (%) was found during S4 in Sample P. The highest % of ERR was observed in the silkworms reared using *R. communis* (Sample C) (85.51%) while the lowest ERR (%) was found in silkworms reared using *C. papaya* (Sample P). Thangavelu and Phukon (1983) also observed variation in ERR in response to host plants in their study in *S. ricini* and recorded ERR (%) of 77.5%, 79.5%, 74.5% and 65% respectively on castor, kesseru, tapioca and barkesseru. Nangia et al. (2000) also reported highest percent of ERR (94.65%) on castor followed by tapioca (77.25%), barkesseru (77.20%), gulancha (64.8%) and the least ERR% was observed in the silkworms reared using papaya (61.49%).

The emergence rate (%) however was highest (91.75%) during S3 in *R. communis* fed silkworms (Sample C). It was found to be higher during S1 for *M. esculenta* (Sample T), and *G. arborea* fed silkworms (Sample G) and S2 for *H. fragrans* (Sample K)*,* and *C. papaya* fed silkworms (Sample P). The lowest ER% (76.57%) was observed in silkworms reared using *C. papaya* leaves. The difference in emergence rate (%) may be due to the variation of nutritional content of the host plants during different seasons. Birari et al. (2019b) also suggested that the rearing performance of *S. ricini* is influenced by the host plants used for rearing. They also reported that castor exhibited highest larval weight, survivability, emergence rate and effective rate of rearing as compared to the silkworm reared using different host plants like tapioca, adruso, banyan tree and Indian almond. Jaiswal and Kumar (2005) also reported that the economic characters of the silkworm, *S. ricini* are not only controlled by genes but are known to be influenced by different climatic factors such as temperature, humidity, and photoperiod etc.

The survival ratio of the present study was found to be in the range of 71.71±7.92 to 90.74±4.73% based on the host plant and seasonal variation. The highest survival ratio was observed in the silkworms reared using *R. communis* leaves (Sample C) during Season 3 while the lowest was observed in the silkworms reared using *C. papaya* leaves (Sample P) during Season 4. The survival ratio was found to be highest during Season 3 for the silkworms reared using *R. communis* (Sample C), *M. esculenta* (Sample T) and *H. fragrans* leaves (Sample K) while the silkworms reared using *G. arborea* (Sample G) and *C. papaya* (Sample P) leaves showed highest survival ratio during S1. The survival ratio (%) of eri silkworm is known to be influenced by several factors, including host plant and seasonal variations. The nutritional content of the food plant, particularly protein and other essential nutrients, can affect the growth, development, and survival of the silkworms. Other than that, temperature also plays an important factor in the growth and survival of the silkworms. Extreme temperatures can decrease their growth and survival rates. Humidity also impacts the survival of silkworms. Therefore, optimal temperature is necessary for the proper growth, development, and survival of *S. ricini.* Rahmathulla (2012) also reviewed that the silkworms being cold-blooded animals, is directly affected by temperature on various physiological activities. It was also mentioned that the temperature has direct correlation with the growth of silkworms and that the survival, duration of life cycle is directly affected by the rise in temperature.

The silk gland distributed throughout the length of the body produces the cocoon, and the growth and development of the silk gland is dependent on the healthy silkworm and nutritional state (Kumar and Gangwar, 2010; Sarmah et al., 2011). The cocoon weight indicates the yield attributes as it is the ultimate product of sericulture.

In this study, it was observed that the cocoon weight was higher in the silkworms reared using *R. communis* (Sample C), *M. esculenta* (Sample T) and *H. fragrans* leaves (Sample K) while the silkworms reared using *C. papaya* (Sample P) showed lower cocoon weight. The cocoon weight was found to be highest during Season 1 for *R. communis* (Sample C) and *H. fragrans* fed silkworms (Sample K) while the silkworms reared using *M. esculenta* (Sample T), *G. arborea* (Sample G) and *C. papaya* (Sample P) was found to be highest during Season 3.

The present findings are comparable with that of Chaudhary (1979); Sarmah et al. (2002) and Patil et al. (2009) who observed the variations in cocoon weight when silkworms were reared using different host plants and genotypes. They suggested that cocoon weight varied with types of host plant provided during the larval stage of the silkworms. Similar observation was recorded by Devaiah and Dayashankar (1982) and Shifa et al. (2014) who reported variation in shell weight and opined that the shell weight varied with host plants provided at the larval stage.

The shell weights of the silkworms were also observed to vary significantly depending on the host plant and seasonal differences. The highest shell weight was observed in the silkworms reared using *R. communis* (Sample C) while the lowest was observed in the silkworms reared using *C. papaya* leaves (Sample P). The shell weight of the silkworms reared using all the selected host plants (Sample C, Sample T, Sample G, Sample K, Sample P) was found to be higher during S1 and S3 while it was found to be lowest during Season 4.

The shell ratio (%) was also found to follow similar trend as the cocoon and shell weight and was found to be higher in *R. communis* fed silkworms (Sample C) followed by *H. fragrans* (Sample K) and *M. esculenta* fed silkworms (Sample T). Studies with different host plant genotype by Mandali and Narayanamma (2015) recorded shell ratio of 18.44% when reared with castor genotype PCH111. The finding was in support of the studies of Ramakrishna et al. (2003); Shifa (2016) and Prasanna and Bhargavi (2017) who reported that the shell ratio varies depending on the type of host plants used for feeding the silkworms.

Slansky and Scriber (1985) also proved that a good diet promotes silkworm growth, which immediately improves silk quality. In comparison to tapioca, castor is thought to be the best primary host plant species for eri silkworm. Basaiah (1988); Jayaramaiah and Sannappa (1998); Dutta and Khanikor (2005); Chakravorty and Neog (2006); and Singh and Goswami (2012) have also reported that the foliar constituents of castor have a positive correlation with cocoon parameters such as cocoon weight, shell weight and shell ratio (%). Murugan et al. (1998) and Alipanah et al. (2020) also reported similar results on direct influence on silkworm health. The variation in seasons may also plays a crucial role as it influences the temperature, humidity, rainfall which indirectly affects the growth and development of eri silkworm.

From the present study, it was evident that *R. communis* stands out as the primary and most suitable host plant for eri silkworm that provides superior nutrition to the larvae resulting in better growth and silk production. The existence of biochemical components in the leaves such as moisture, protein, phenol, and other nutrients may explain variances in rearing performance and cocoon features. It was also found that the alternative host plants like *M. esculenta, G. arborea, H. fragrans,* and *C. papaya* may also be considered suitable to rear eri silkworms. According to Nangia et al. (2000) who observed that castor (*R. communis*) was the primary host plant, followed by tapioca (*M. esculenta*), kesseru (*H. fragrans*), and papaya (*C. papaya*). Hazarika et al. (2003) also identified castor as the best host plant for *S. ricini* based on leaf nutritive value. The nutrients influence all aspects of rearing performance of the insect and give maximum potential fitness, castor can be considered as a better food plant for Eri silkworm (Debaraj et al., 2003).

5.3 Study on biochemical parameters of *S. ricini* fed on different host plants

The protein concentration of the haemolymph of *S. ricini* was found to be highest in the silkworms reared using *H. fragrans* (Sample K) whereas the lowest protein concentration was found in the haemolymph of the silkworms reared using *C. papaya* leaves (Sample P). The haemolymph protein concentration of the larvae of *S. ricini* was at the highest peak during the fifth instar stage. According to Srivastava et al. (1982), nutrition has a crucial role in the growth, development and metamorphosis particularly in lepidoptera where the adult is a non-feeding stage. Kaleemurrahman and Gowri (1982) found total protein levels higher in castor than in borkesseru. Similar observations were also recorded by Banno et al. (1993); Murthy et al. (2014) where the protein concentration was found to be at the highest concentration at the end of larval stage, during fifth instar stage of the silkworms.

Banno et al. (1993) and Aruga (1994) found that high haemolymph protein concentrations are linked to high mulberry leaf consumption and, as a result, a high rate of conversion and accumulation in *B. mori* hemolymph. Chen (1978) also stated that the fat body of the larva is involved in the synthesis and secretion of haemolymph protein during the larval growth however, when the larva stops feeding these proteins are removed from the haemolymph and stored as intracellular granules to be used during the time of metamorphosis. The protein concentration is an indicator of metabolic activity. The haemolymph protein content is also influenced by the quality and nutritional content of the food plants. Several studies have been carried out on the chemical constituents observed in the food plants of eri silkworms (Pathak, 1988 and Shaw, 1998). The research conducted by Merenjungla and Kakati (2013) examined how the nutritional levels in young, semi-tender, and mature leaves of four host plants (castor, tapioca, payam, and kesseru) of eri silkworm in Nagaland vary across the seasons. Out of the four plants that an eri silkworm fed, they found castor had the highest protein content.

Similar trend was observed in the haemolymph carbohydrate concentration. The carbohydrate concentration of the haemolymph of *S. ricini* was found to be highest in the silkworms reared using *H. fragrans* (Sample K) whereas the lowest carbohydrate concentration was found in the haemolymph of the silkworms reared using *C. papaya* leaves (Sample P). According to Chippendale (1978), a mature non-feeding larva typically accumulates the most carbohydrates in preparation for transformation which is mostly stored in the fat body as glycogen and trehalose in the haemolymph. Glycogen and trehalose supply glucose during metamorphosis, which serves as an energy source and a substrate for the creation of pupal and adult cuticle. Simex and Kodrik (1986) also found the carbohydrate content was highest during the last instar. Throughout larval development, large concentrations of carbohydrates in hemolymph are retained as an energy store to be used later during metamorphosis, pupal, and adult stages (Mishra et al., 2010).

Carbohydrates are one of the primary elements of nutrition that are used as an energy source for all critical vital processes, either directly or indirectly. The level of carbohydrates during larval development reflected the extent to which carbohydrates, the principal sources of energy in the body for larval growth and development, were utilised. This eventually influences the difference in silk production quality and quantity (Kumar and Michael, 2012). Climate, as well as dietary materials, is significant regulating factors for protein, carbohydrate, reducing sugar, and lipid content of haemolymph and silk gland, which are essential for larval growth and metamorphosis stages, according to Chhatria et al. (2018).

The mineral content is critical for their growth, development, and silk production. In the present study, the mineral composition of silkworms reared using different food plants was investigated, with a focus on sodium (Na), magnesium (Mg), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) concentrations. The results revealed significant differences across the groups, showing the influence of food plants on the mineral content of silkworms showed the highest quantities of Na, Mg, K, Ca, Mn, Fe, Cu, and Zn, indicating that the nutritional content of the host plant has resulted in an overall enrichment of minerals in the silkworms. The mineral content in the diet of Eri silkworms is typically supplied through the consumption of food plants. Research on the mineral content of eri silkworms has revealed high level of potassium, iron, manganese, and zinc in both prepupae and pupae (Ray and Gangopadhyay, 2021).

The nutritional requirements of Eri silkworms, like those of other silkworm species, vary depending on the environment and mineral availability. Several important minerals play an important role in their physiological activities. Liu (1996) stated that most of the trace elements present in the pupae of *A. pernyi* were necessary trace elements. Calcium required for the creation of the silkworm's body structure and plays an important part in the molting process. Phosphorus essential for growth since it is required for energy metabolism and the production of nucleic acids. Potassium regulates osmoregulation and turgor pressure, which contributes to water balance. Magnesium, a cofactor for enzymes engaged in metabolic pathways. Sodium and chlorine for osmoregulation and fluid balance. Iron, essential for hemoglobin, aids in oxygen transfer. Zinc, an enzyme cofactor, required for growth and development, whereas copper assists in hemocyanin synthesis, which facilitates oxygen delivery in the hemolymph. The intricate interplay of these minerals emphasizes their importance in molting and silk-producing capabilities.

The selection of host plants plays a critical role in determining both the quality of silk produced and the overall efficiency of the silk farming process. Various biochemical parameters such as protein, carbohydrate, lipid content, and the presence of secondary metabolites influence the growth, development, and silk yield of the silkworms. Understanding these biochemical influences provides insights into the practical implications for sericulture operations, enabling farmers to optimize conditions for higher productivity and better quality silk (Sengupta et al., 2021).

One of the most important biochemical parameters influenced by the host plant is the protein content of the diet, as proteins serve as the fundamental building blocks for silk production, specifically contributing to the synthesis of fibroin and sericin (Balamurugan & Chandrashekar, 2022).Carbohydrates are another essential biochemical factor that plays a significant role in the growth and development of *S. ricini* which also serves as the primary source of energy, fuelling cellular activities and metabolic processes required for growth and cocoon formation. Host plants with higher carbohydrate levels promote faster larval growth and shorter developmental periods, which can, in turn, reduce the overall time required for silk production cycles (Sinha et al., 2020). Minerals, though required in smaller amounts, are equally essential. They play a role in metabolic processes and the overall health of the silkworms (Rahman et al., 2019). This is particularly advantageous for farmers looking to maximize the number of silk harvests per year. By selecting the most suitable host plants based on biochemical factors, farmers can improve productivity, reduce costs, and ensure the production of high-quality silk (Sharma & Debnath, 2021).

5.4 Study on the nutritional content of *S. ricini* fed on different host plants

Proximate analysis of *S. ricini*

The proximate analysis of *S. ricini* fed on different host plants was done. It was found that the silkworm contains 3.31 to 5.56 % crude fibre, 9.04-10.58% nitrogen, 11.02 to 14.14% total fat, 4-6% ash, and 56-60% protein. The present findings were at par with the findings of Mishra et al. (2003), who found that the proximate compositions (%) of non-mulberry and mulberry silkworm pupae were within the range of total protein (12 to 16%), total fat (11 to 20%), carbohydrate (1.2 to 1.8%), moisture (65 to 70%), and ash (0.8 to 1.4%). Zhou and Han, (2006) also recorded that the pupal powder of *A. pernyi* had a moisture content of 7.6%, crude protein content of 71.9%, fat content of 20.1%, and ash content of 4.0% on a dry matter basis. Longvah et al. (2011) also found that the pre-pupae and pupae of the Eri silkworm were good sources of protein (16g %), fat (8 g%) and nutrients.

The study on the proximate analysis of the larval extract of *S. ricini* and leaf extract of the host plants used for rearing the silkworms further elucidates the relationship between nutritional composition of the larvae and its dietary source. These findings also suggested that the nutritional composition of *S. ricini* larvae were influenced by the nutritional content of their host plants. Therefore, selecting appropriate host plants with favourable nutritional profile is crucial for enhancing the nutritional content of the larvae of *S. ricini* which in turn can potentially improve the suitability of the silkworm for various applications in producing quality silk and value added products.

Amino acid profiling of *S. ricini*

The present study on the amino acid profiling of *S. ricini* had identified many amino acids. However, the amount of each amino acid found varied based on the type of host plant used. Threonine, lysine, and methionine were indispensable amino acids that play a vital role in promoting growth and development. Furthermore, these amino acids were found in varied amounts in the larvae depending on the host plants they were fed on. This finding was at par with results of Zhou and Han (2006) who discovered that the pupal protein contains 18 known amino acids, including all required amino acids and Sulphur-containing amino acids. The pupal protein was of high quality when compared to the amino acid profile recommended by FAO/WHO due to its high amount of essential amino acids. According to Longvah et al. (2011), the amino acid scores of Eri pre-pupae and pupae protein were 99 and 100, respectively, with leucine serving as the limiting amino acid in both cases. The net protein utilization (NPU) of pre-pupae and pupae was 41 against 62 in casein. The protein digestibility corrected amino acid score (PDCAAS) was 86. Amino acids, being the fundamental constituents of proteins, have a crucial function in the growth, development, and general well-being of silkworms. Amino acids are necessary for the process of protein synthesis, particularly during the larval stage, when there is a rapid increase in size, development, and shedding of the outer skin. This ensures the production of proteins that are needed for different stages in the life cycle (Kakutani et al., 2011). Amino acid facilitates constriction of blood vessels and clot (Clandinin et al., 1997).

The economic significance of Eri silkworms in silk production is strongly linked to amino acids. Amino acids are necessary to produce silk proteins, specifically sericin and fibroin, which are the primary components of silk. Aside from their involvement in protein synthesis, amino acids help with energy metabolism by acting as a potential energy source and engaging in metabolic processes like the tricarboxylic acid (TCA) cycle. Amino acids also have an impact on immune system support, with specific amino acids such glycine, glutamine, and arginine helping the silkworm resist infections and illnesses (Paul and Dey, 2014).

Essential amino acids (EAAs) like threonine, lysine, and methionine play a pivotal role in the growth and development of silkworms, particularly in *Samia ricini*. These amino acids are vital for various physiological functions, including protein synthesis, metabolic processes, and overall health. Threonine, for instance, is crucial for the synthesis of proteins, acting as a precursor for key structural proteins necessary for the formation of silk fibroin, which is essential for cocoon production. Furthermore, threonine aids in amino acid metabolism and helps maintain nitrogen balance in the body, which is critical for optimal growth (Jiang et al., 2021).

Lysine is another important EAA that significantly influences silkworm growth. It serves as a building block for proteins and is essential for tissue growth, including muscle development. Adequate lysine levels are vital for promoting weight gain in silkworms and are directly linked to silk protein synthesis. Research has shown that insufficient lysine can lead to reduced silk yield and quality, emphasizing its importance in sericulture (Sharma et al., 2020). Additionally, lysine is involved in hormone regulation, which affects various growth and developmental processes throughout the silkworm's life cycle.

Methionine also contributes significantly to the growth and health of silkworms. It acts as a methyl donor in numerous biochemical processes and is essential for the synthesis of cysteine, another critical amino acid. Methionine possesses antioxidant properties that help detoxify harmful substances within the silkworm's body, thereby promoting overall health and longevity. Moreover, adequate levels of methionine support optimal growth rates and enhance feed efficiency, ultimately leading to better larval development and higher survival rates (Ali et al., 2019).

Furthermore, amino acids are essential for nutrient transfer inside the organism, and particular amino acids may act as store molecules when food is scarce. Another key function is enzyme activation, which requires amino acids as cofactors or substrates for biochemical reactions that govern metabolic pathways in the silkworm. Amino acids facilitate antioxidant defence, with amino acids like cysteine and methionine playing a crucial protective role against oxidative stress.

5.5 Antioxidant and scavenging activity of larvae of *S. ricini* fed on different host plants

Antioxidant activity is crucial to silkworm health and immunity, acting as a defensive mechanism against the harmful effects of oxidative stress. Silkworms, like all aerobic creatures, produce reactive oxygen species (ROS) as a byproduct of metabolic processes. While these molecules can operate as signalling agents at low concentrations, excessive ROS accumulation causes oxidative stress, which can harm cellular components such as lipids, proteins, and DNA (Li et. al. 2018). The antioxidant and scavenging activity of methanolic extract of *S. ricini* larvae was studied through standard antioxidant assays like DPPH, ABTS and Ferric Reducing Antioxidant Power assay. The IC_{50} values of DPPH radical scavenging activity of larval extract of *S. ricini* was seen to vary depending on the host plant. The IC_{50} value of silkworms reared using *R. communis* (Sample C) was lowest at 26.85±0.54µg/mL while that of silkworms reared using *C. papaya* (Sample P) was highest at 51.93±0.31µg/mL.

The finding was at par with the finding of Deori et al. (2014) who observed that the IC_{50} value of Muga silkworm pupal extract was 25.83µg/mL. The eri silkworm pupae powder exhibited a significant DPPH scavenging activity, with an IC_{50} value of 60.58µg/mL (Pachiappan et al., 2016). Ghosh et al. (2020) found that the reeling waste pupae of mulberry, tasar, and muga silkworms, which are byproducts of the silk industry, exhibited potent antioxidant capabilities. The IC_{50} values for these byproducts were 28.40µg/mL, 29.31µg/mL, and 40.74µg/mL, respectively.

The antioxidants found in the larval extract of eri silkworms inhibit the creation of free radicals by providing protons to the DPPH radical. As a result, the absorption of scavenging activity gradually decreases (Deori et al., 2014). The reduction in absorbance at a wavelength of 517 nm served as an indicator of the degree to which the radical was scavenged. The relative stability of DPPH has made it a widely used method to assess the free radical scavenging activity of various substances (Jha et al., 2014). A lower IC_{50} value signifies a greater capacity to scavenge free radicals. The analysis of percent inhibition indicates that the larval extract of eri silkworm scavenge DPPH free radicals in a manner that is dependent on the concentration. The variation in the IC_{50} value of the silkworm reared using different host plants observed could be due to the difference in the nutritional content of the host plants. The percent inhibition of the DPPH scavenging revealed that the DPPH free radical was scavenged by *S. ricini* in a concentration dependent manner.

The (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) ABTS assay was performed to assess the scavenging capacity of *S. ricini* fed on different host plants. The IC_{50} value of ABTS scavenging capacity was found to be lowest in the silkworms reared using *R. communis* (Sample C) while the highest was recorded in the silkworms reared using *C. papaya* (Sample P). Host plants being the only source of nutrition for the silkworm are also known to contain various phytochemicals and antioxidant properties therefore, these could attribute to the overall antioxidant capacity of the silkworm and, the differences in the ABTS scavenging capacity of eri silkworms reared using different food plants could be due to the variations in the nutritional content of the host plants. Studies by Zhang et al. (2021) and Cermeño et al. (2022) also suggests that the silkworm pupae contain many polyphenols and peptides with antioxidant properties which are effective at scavenging ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and DPPH.

The variation in the enzyme activity is observed may be due to the variation in the nutritional and phytochemical content of the host plants which might have induced stress in the physiology of the silkworm. Antioxidants are a distinct category of compounds that possess the ability to prevent the process of oxidation. Oxidation is generally regarded as the process that triggers a chemical response and produces free radicals within the body. These volatile free radicals are responsible for cellular damage, which can result in many illnesses. Recently, there has been a significant increase in interest regarding natural antioxidants as food and health supplements. This is due to their potential to decrease the likelihood of various disease outbreaks in the human body, without causing any harmful effects (Aruoma, 2003). Studies suggest that silkworm including *S. ricini* and *B. mori* possesses free radical scavenging activity that helps to protect cells from oxidative damages. The antioxidant activity of silkworm also suggests potential health benefits for humans.

In silkworms, oxidative damage can have a negative impact on health, growth, immunity, and silk output. As a result, preserving their overall well-being requires a strong antioxidant defence. Antioxidants not only safeguard cellular health, but they also play an important part in silkworm immunity. Silkworms' immune system is mostly innate, relying on cellular and humoral responses to fight diseases. When silkworms are exposed to pathogens such as bacteria or viruses, their immune cells, known as haemocytes, release ROS as part of their defence mechanism. The respiratory burst is a procedure that helps to eliminate infecting diseases. However, excessive ROS production might injure the host's own tissues if not controlled appropriately. Antioxidants serve to regulate this immune response by regulating ROS levels, minimizing self-inflicted oxidative damage and allowing the immune system to stay effective in eliminating infections.

Antioxidants also improve metabolic efficiency, especially during the physiologically demanding processes of moulting and metamorphosis. Because of the increased metabolic activity, these processes produce large amounts of ROS. Antioxidants help to reduce the oxidative stress associated with these stages, promoting appropriate development and growth (Park et. al., 2022). Furthermore, antioxidants increase the silkworm's resistance to environmental challenges such as temperature changes and pollution, assisting in the maintenance of health under bad conditions. Furthermore, antioxidant activity plays a significant role in silk production. Oxidative stress can affect the amount and quality of silk produced. By maintaining the health of the silk glands, antioxidants ensure that *S. ricini* produces high-quality silk, contributing to the economic success of sericulture. Overall, antioxidant activity is essential for protecting silkworm health, supporting immune function, and ensuring robust silk production.

5.6 Immunological response of *S. ricini* fed on different host plants

The immunological response of *S. ricini*is a critical aspect of the overall health and immunity of the silkworm. In the present study, the immune response of the silkworms fed in different host plants were assessed by evaluating the activities of two key enzymes: Glutathione S-Transferase (GST) and Catalase. These enzymes play significant roles in detoxification processes and oxidative stress responses, respectively.

Glutathione S-Transferase (GST)

The GST activity was found higher in the silkworms reared using *C. papaya* leaves (Sample P) while the lowest activity was observed in the silkworms reared using *R. communis* leaves (Sample C). The increased enzyme activity because of the biochemical content of the host plant might have caused stress in the silkworm due to which the silkworm showed higher enzyme active in response.

Insects have a broad spectrum of immune mechanism to fight against the invading foreign particles even though they do not have complicated immunoglobulins. The glutathione s-transferase enzyme serves as an essential antioxidant and plays an important role in the immunity of silkworms. It helps to neutralise the harmful reactive oxygen species (ROS) that is generated during immune response (Mahanta et al., 2023).

Catalase

The catalase activity was highest in the silkworms reared using *R. communis* (Sample C) while the lowest was observed in the silkworm fed with *C. papaya* leaves (Sample P). The reason for the decrease in catalase enzyme activity in *C. papaya* fed silkworms (Sample P) could be due to reduced intake of food and metabolic changes. The *R. communis* fed silkworms (Sample C) exhibited an increased level of immunity compared to the other groups studied, shown by the elevated activity of the antioxidant enzyme catalase. Increased activity of antioxidant enzymes can help strengthen the defence system of the silkworm against diseases that indicates a higher level of health in the host organism. The catalase enzyme is essential for defence mechanism of insects and is necessary during interactions with pathogens. It is an essential antioxidant enzyme that reduces oxidative stress and protects the cell against damage caused by oxidation (Bandyopadhyay et al., 1999). Hao et al. (2003) established that Reactive Oxygen Species (ROS) are crucial in the non-specific defence mechanism.

Kumar et al. (2003) also found that the catalase enzyme enhances signal transduction and promotes many biochemical events, including cell development and death. The pathogen-induced stress can elevate ROS levels, leading to the breakdown of cell structures and normal cellular signaling pathways. Catalase possesses the most rapid catalytic activity, as it may decompose millions of hydrogen peroxide molecules into water and oxygen in a single second. The regular metabolic processes produce a harmful by-product called Hydrogen peroxide (H_2O_2), which must be promptly decomposed into oxygen and water which otherwise can result in damage to cells, tissues, and important macromolecules such as DNA, proteins, and lipids.

Overall, the study assessed the impact of different host plants on the growth, nutrition, and immune health of *S. ricini* silkworms, particularly focusing on *R. communis, M. esculenta, G. arborea, H. fragrans*, and *C. papaya*. *R. communis* (castor) consistently provided the most favorable outcomes for silkworm growth and development. Silkworms reared on castor (Sample C) experienced shorter larval and pupal durations, higher cocoon weight, and the highest effective rearing rate (ERR), indicating more efficient development compared to silkworms reared on other plants, such as *C. papaya*, which extended the developmental period.

In terms of nutrition, silkworms fed on *H. fragrans* showed the highest hemolymph protein concentration, an indicator of health and silk productivity, though *R. communis* offered balanced nutrient levels, including the highest mineral content (calcium and magnesium), considered essential for metabolism and silk production. Again, in terms of immune responses varied with diet, *C. papaya* induced higher GST activity, most likely caused due to stress, while *R. communis* fed larvae had higher catalase levels, indicating better oxidative stress management. Antioxidant assays (DPPH, ABTS) confirmed stronger defenses in *R. communis* fed larvae.

So, the present study proposes that *R. communis* is the ideal host plant for *S. ricini*, yielding optimal growth, cocoon quality, nutrition, and immunity. However, *H. fragrans* and *M. esculenta* serve as effective secondary hosts, supporting healthy silk production when *R. communis* is scarce.