CHAPTER-2

REVIEW OF LITERATURE

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2.1. Ericulture and its practice in North-eastern part of India

The North-eastern region of India is widely recognized as a biodiversity hotspot due to its abundance of diverse and valuable bio-resources. This region is particularly significant in the production of various types of silk. It is home to a variety of silkworms and their host plants, including Muga (*Antheraea assamensis*), Mulberry (*Bombyx mori*), Eri (*Samia ricini*), Tasar (*Antheraea mylitta*) and Oak Tasar (*Antheraea proylei*) (Singh *et al*., 2017).

Kalita and Dutta (2014) in their study documented the presence of 12 species of silk-producing insects from two different insect families in Assam from the region. In a more recent study conducted by Boro and Borah (2020), a remarkable 31 species of saturniidae and 9 species of bombycidae were identified in the region, highlighting the region as a veritable hotspot for diverse flora and fauna.

Ericulture, an integral part of sericulture is particularly the rearing of Eri silkworm (*S. ricini*) and the cultivation of its host plants for the production of Eri silk, which is a type of non-mulberry silk, typically practiced by the farmers of the society in the North-eastern region of India. Chowdhury (1982 and 1984), observed that, Ericulture is the primary form of sericulture in this region and is not only a traditional practice but also an important part of the local culture and way of life. According to Gargi *et al.* (1994), the practice of sericulture has been in existence since ancient times.

As per the information provided by the Directorate of Sericulture under the Government of Assam, Eri culture is widespread across numerous districts of the state, namely Goalpara, Lakhimpur, Kamrup, Jorhat, Dibrugarh, Cachar, Udalguri, Morigaon, Darrang, Nagaon, Karbi Anglong, Kokrajhar, Golaghat, NC Hills, and Dhubri. These districts collectively contribute to about 65% of the total Eri silk production in India.

Teotia and Bajpeyi (2009) discovered that Eri silkworm rearing is a prevalent practice in the Bodo community of Kokrajhar District, and it is not just for producing silk but also for enjoying the pre-pupae/pupa as a delicacy. The Bodo community considers Eri rearing to be a household activity.

Sarmah (2011) has referred to Eri silk as the "poor man's" silk due to its relatively simple production process that requires minimal infrastructure and the fact that it is commonly practiced by the economically disadvantaged members of society. As per the report of Sarmah (2011), indigenous communities in the north eastern region of India has discovered a gastronomic use for the Eri silkworm: the pupae are a source of delicacy. The practice of utilizing silkworms as a food source has been carried out and documented in many countries worldwide. For the tribes of the region the Eri chrysalis is a highly delectable dish, whereas the cocoon is seen as a secondary product. Sarmah *et al.* (2012) also suggested that the Brahmaputra valley of Assam, along with its adjacent foothills, is likely the native habitat of the Eri silkworm, scientifically known as *S. ricini*.

Brahma (2015) conducted a study in the Kokrajhar district of Assam, which revealed that the tribal population in the area has been practicing Ericulture for generations, and it is deeply rooted in their culture and tradition, particularly among the Bodo tribes. Approximately 60% of women in the region are involved in ericulture, which provides a source of income for their families.

Harapanahalli and Raaja (2016) found that Eri culture is a vital component of the rural economy, particularly for women in rural and tribal communities. These women utilize their idle time to rear Eri silkworms and weave Eri fabrics using their traditional knowledge, thereby generating economic activity in their communities.

In a more recent study by Parameswaranaik *et al.* (2020), it was reported that eri silkworm rearing has become a traditional occupation for people living in the Bodoland Territorial Council (BTC) area of Assam. The study found that around 44% of respondents were involved in ericulture, with a high level of entrepreneurship development. Gogoi *et al.* (2023b) studies in Dhemaji district of Assam also explained that sericulture is practiced in large scale almost in every village from the ancient time.

In terms of silk production, the North- Eastern region of India has been found to be a profitable destination for the production of Eri silk. As per the report by Singh *et al.* (2017) the north-eastern states of India are responsible for approximately 99% of the total Eri silk production in the country. The raw silk production of Eri silkworm constitutes a significant proportion of 62.88% in the non-mulberry category, accounting for 8038 Matric Ton during 2015-2016 in India (Singh and Ahmed, 2017). This proportion has since increased to 70.19% in the 2020–21 periods, constituting a significant portion of the overall non-mulberry raw silk production in India (Jigyasu *et al*., 2022). The versatility of Eri silk with respect to its thermal properties, ability to mix well with other types of fibres, pupae containing high levels of nourishing protein and guaranteed crop yield has made it an attractive option for livelihood opportunities also in non-traditional states of the country.

2.2. Food plants of Eri silkworm and their effect on eri silkworm

Eri silkworms are known for their versatility when it comes to feeding habits, as they can consume a wide range of food plants. Several researchers have reported using various host plants for Eri silkworm, including Castor (*R. communis*), Kesseru (*H. fragrans*), Tapioca (*M. esculenta*), Papaya (*C. papaya*), Jatropha (*J. curcas*), Barpat (*A. grandis*), and Payam (*E. fraxinifolia*) (Singh and Das, 2006; Chakravorty and Neog, 2006; Bhattacharya *et al.,* 2006, Das *et al.,* 2006; Chowdhary, 2006 and Bindroo *et al.*, 2007).

Reddy *et al*. (1989) found that Eri silkworms have a wide range of host plants preferences, feeding on more than 29 different types of plants. Sharma *et al.* (2002) observed that NBR-1 Castor variety showed optimal capacity for superior quality cocoon yield in Eri silkworm.

According to Bindroo *et al.* (2007), a total of 24 plant species have been identified as suitable hosts for eri silkworm. These plants have been categorized as primary, secondary, and tertiary based on how frequently they are utilized and how palatable they are to the moth.

Kumar and Elangovan (2010) conducted a study using Castor (*R. communis*), Tapioca (*M. utilissima*), Jatropha (*Jatropha curcas*), and Papaya (*Carica papaya*) as host plants for Eri silkworm rearing. The larvae fed with castor leaves produced the highest yield of silk. Kumar and Gangwar (2010) also reported the use of Castor (*R. communis*), Tapioca (*M. utilissima*), Barara (*J. curcas*), and Papaya (*C. papaya*) as host plants for Eri silkworm rearing. Rajudarai *et al.* (2010) found castor leaves to be superior to tapioca leaves in all seasonal conditions for Eri silkworm rearing.

Similarly, Deka *et al.* (2011) investigated the impact of three different food plants (castor, kesseru, and tapioca) on the growth parameters of Eri silkworms. The results of their study indicated that the red variety of castor was the most effective food plant in promoting larval growth and improving spinning characteristics. In contrast, kesseru and tapioca were found to be less effective in promoting growth and spinning characteristics. Moreover, the study found that growth parameters were better during the spring season, followed by autumn, winter, and summer. These findings could have significant implications for farmers and policymakers in determining the optimal feeding strategies for Eri silkworms and maximizing silk production.

Subramanianan *et al.* (2013) experimented with 10 different food plants for feeding the fifth instar larvae of Eri silkworm after a pre-starvation period of 30 min under laboratory conditions, which included *Calotropis gigantean*, *Nerium odourm*, *Leucaena leucocephla*, *Parthenium hysterophororum*, *Annona squamosa, Pongamia pinnata*, Coconut leaf, banana leaf*, Sesbania grandiflora*, and *Terminalia catapa*. Additionally, Venu and Munirajappa (2013) reported the use of tapioca as a secondary food plant along with castor for commercial rearing of Eri silkworm larvae.

Chutia *et al.* (2014) reported the use of alternative food plants such as barpat, borkesseru, tapioca, and payam for Eri silkworm rearing. Acceptance of *C. papaya* plant leaves for Eri silkworm was reported by Radhika *et al.* (2017) they found that *C. papaya* can be used as a tertiary food plant for Eri silkworm, particularly during the shortage of primary food plant castor. This can be a good option for the successful production of Eri silk.

Naik and Murthy (2014) conducted research on the use of new host plants for Eri silkworm, including Fountain tree, banyan tree, Indian almond and Carrot leaves. Results indicated that only carrot leaves were found to be a suitable host plant, while the others were not as effective.

In a study conducted by Saikia and Yadav (2015), the effects of five host plants, namely castor, kesseru, borkesseru, tapioca, and gulancha on the larval and cocoon characteristics of Eri silkworm were investigated. The results indicated that castor was the most superior host plant followed by borkesseru, tapioca, kesseru, and gulancha in terms of their economic characteristics. The study also suggested that tapioca leaves could be used as an alternative feed during castor off-season.

Sharma *et al.* (2015) conducted a study to evaluate the impact of different food plants, including castor (*R. communis*), kesseru (*H. fragrans*), barpat (*A. grandis*), and borkesseru (*A. excelsa*), on the economic traits of the C2 breed of Eri silkworm developed by the Central Silk Board, CMER&TI, Lahdoigarh. Their findings indicated positive economic traits in the C2 breed when fed with these food plants.

Ahmed *et al.* (2015) compared the effects of using different perennial food plants on the rearing performance of Eri silkworms. They found that combining castor with barpat leaves during the early stages of silkworm rearing resulted in superior larval performance compared to feeding on kesseru leaves alone from the first to the fifth instars stage. The result suggested the use of barpat food plants for rearing Eri silkworms, which could potentially overcome the problem of non-availability of sufficient leaves throughout the year and aid in commercializing Ericulture.

Several studies have been conducted to determine suitable food plants for rearing Eri silkworms. Cassava leaves have been found to be a viable option for Eri silk production in Tamil Nadu, with potential for poverty alleviation (Sakthivel *et al.,* 2016).

According to Kumar *et al*. (2017) farmers who raise Eri silkworms often rely on plants from several different families, including Euphorbiaceae, Araliaceae, Rutaceae, Simaroubaceae, and Apocynaceae. Castor (*R. communis*) and Kesseru (*H. fragrans*) are two primary host plants that are commonly used for their rearing, while Tapioca (*M. utilissima*) is also used as a secondary food source.

Deuri *et al.* (2017) reported Castor and Tapioca as most promising host plants adapted by the rearers for rearing of Eri silkworms in the BTC region of Assam. Similarly, Baruah (2012) reported the successful rearing of Eri silkworms on castor and kesseru food plants. These findings could have important implications for the commercial rearing of Eri silkworms and provide insights for farmers and policymakers in selecting the most appropriate food plants for Eri silkworm rearing.

Castor has been identified as a primary food plant for Eri silkworm rearing, with high economic parameters (Pallabi and Sharma, 2017). Several studies have been conducted on the use of different food plants for the rearing of Eri silkworm. Castor (*R. communis*) and Kesseru (*H. fragrans*) have been identified as the primary host plants, while Tapioca (*M. utilissima*) is commonly used as a secondary food plant (Singh *et al.*, 2017).

Similarly, Birari *et al.* (2019) studied the use of different host plants, including castor, tapioca, arduso, banyan tree and Indian almond, for rearing Eri silkworms in laboratory conditions. The study revealed that castor was the most effective food plant for rearing Eri silkworms.

Priyadarshini and Kumar (2019) also reported the potential use of castor, tapioca, and Payam food plants for rearing Eri silkworms. The use of various host plants for ericulture has become a promising approach towards sustainable ericulture with positive outcomes.

Similarly, Swatiga *et al.* (2019) evaluated different castor genotypes, including GCH 4, GCH 7, DCH 519, and TMV 5, for their suitability with the C2 Eri silkworm breed. The results revealed that GCH 4 was the most suitable variety for high yield and recommended it to castor farmers.

Additionally, the Mishing tribes of Assam have successfully used six species of food plants, including *C. papaya*, *G. arborea*, *H. fragrans*, *L. parviflora*, *M. esculenta*, and *R. communis*, in their home gardens for Eri silkworm rearing (Dutta *et al*., 2020).

In a recent study by Narzary & Brahma (2021) has shown that *G. arborea* leaves have potential as food plants for Eri silkworms under rearing conditions in Kokrajhar district. Additionally researchers have identified a range of host plant species that can be utilized for the rearing of Eri silkworm.

In another study, Borah *et al.* (2021) evaluated the effect of two species of Ailanthus, *A. grandis* (Barpat) and *A. excels* (Borkesseru), under different seasonal conditions on the cocoon parameters of Eri silkworm. These findings provide valuable information for the selection of suitable food plants for Eri silkworm rearing, which is important for increasing the productivity and economic benefits of this sector.

Several studies have reported the use of Borpat (*A. grandis*) and castor as suitable food plants for the rearing of Eri silkworms, with potential for upscaling production. Borah *et al.* (2020) investigated the use of these two food plants for rearing different eco-races of Eri silkworm, while Patidar *et al.* (2022) specifically studied the use of Borpat for Ericulture.

Sarkar and Borpuzari (2022) conducted a comparative study to determine the suitability of four major food plants (Castor, Kesseru, Tapioca, and Barkesseru) for commercial Eri silkworm rearing. Their findings indicated that Castor (*R. communis*) was the most suitable and efficient food plant for commercial Eri silkworm rearing and cocoon harvesting, followed by Kesseru (*H. fragrans*), Tapioca (*M. esculenta*), and Barkesseru (*Ailanthus excelsa*). The results of this study could provide valuable insights for farmers and policymakers in developing effective strategies for the commercial production of Eri silk.

2.3. Nutritional composition of host plants

As herbivores, Eri silkworms rely entirely on the leaves of their host plants to obtain nutrients necessary for their growth and development. The host plants have a significant impact on the survival of the silkworms, as well as their rate of food intake, digestion, and assimilation. These factors directly influence the growth and development of the silkworms. The quantity and quality of food intake by Eri silkworm larvae impact various aspects such as growth rate, larval duration, survival rate, and reproductive potential (Das and Das, 2003). The quality of the leaves consumed by the silkworms depends on factors such as moisture, nitrogen, protein, minerals, fibre, sugar, and starch content.

Raychaudhury (1974) claimed that the growth and development of silkworms and their silk production are affected by the quality of the leaves they consume. Although various food plants are consumed by the insects, they are not all equally nutritious. Moisture content in the diet of plant-feeding insects was also highlighted as important by Waldbauer (1968) and Scriber (1978). Scriber and Slansky (1985) stated that the nutritional composition, which comprises the absolute and relative amounts of water, carbohydrates, proteins, amino acids, lipids, fatty acids, vitamins, minerals, etc., is the key factor that determines the quality of food for insects and their performance in terms of growth, development, and reproductive potential. While most of the food plants consumed by the silkworms may contain all the necessary nutrients, the amount of each nutrient may not be properly balanced for optimal growth and development of the larvae (Pathak, 1988).

Yadav and Goswami (1992) reported variations in the crude protein content of silkworm food plants *P. bombycina* and *L. monopetala*. So, it is important of evaluate the biochemical constituents of plant leaves to determine their nutritional value for silkworm rearing. Unni *et al.* (1996 a and b) found that there is a correlation between the growth of Muga silkworms (*A. assama*) and the biochemical constituents of their primary and secondary food plants, particularly in terms of the lipid and fatty acid composition.

Bose and Bindroo (2001) emphasized the significance of protein and amino acids in the diet of silkworm larvae, as they play a crucial role in the synthesis of silk protein. The larval performance was found to be highly dependent on the proportion of protein and carbohydrate in the diet, as reported by Awmack and Leather (2002). Neog *et al.* (2007) also reported variations in the crude protein content of silkworm food plants.

Liu *et al.* (2009) demonstrated that varying nutrient levels in different host plants have an impact on the overwintering of *Helicoverpa armigera* pupae. Sarmah *et al.* (2011) examined the biochemical components of various castor genotypes and their association with the rearing performance of Eri silkworms. They revealed that the biochemical composition of the leaves of different castor accessions affected the efficiency of Eri silkworm rearing.

Neog *et al.* (2011) reported the effect of certain chemical stimulants present in food plants on the feeding and biting behaviour of muga silkworms. Neog *et al.* (2013) found that the Som (*P. bombycina*) plant had a higher proximate value compared to other food plants like Soalu (*L. monopetela*), Mejankari (*L. cubeba*), and Diglotti (*L. salicifolia*). Jyothi *et al.* (2014) recommended the use of mulberry genotypes with higher levels of amino acids, protein, and total phenolics for feeding silkworms to enhance silk productivity.

Similarly, a study by Oftadeh *et al.* (2015) examined the impact of different mulberry plant varieties on the digestive activity and nutritional indices of *Glyphodes pyloalis,* a type of Lepidopteran insect. The results indicated that plasticity in enzyme activity and food utilization is closely related to the cultivar of the host plant. In addition, the physiological and biochemical components of the host plant, such as leaf moisture content, crude fibre, protein and carbohydrate content, as well as the rate of photosynthesis, have been found to have a significant impact on the rearing performance of silkworms (Deka *et al*., 2016).

Deuri *et al.* (2017) conducted a study on the biochemical composition of two commonly used food plants, castor and tapioca. The results showed that castor had a higher content of crude protein (17.43%), lipid (10.95%), and crude fiber (5.53%) compared to tapioca. On the other hand, tapioca had a more balanced composition of primary metabolites such as crude protein (10.83%), carbohydrate (32.14%), and sugar (2.92%), and a lower content of phenol (9.82%) than castor.

The nutritional value of the plant leaves is directly linked to the larvae's preference for food plants. Muga silkworms are primarily reared on the Som (*P. bombycina*) plant, which is considered the best host plant due to its high nutritional value. Ruth *et al.* (2019) conducted a study on the effect of two mulberry plant varieties on silk quality and the production rate of *B. mori*. Their results showed that silk quality is largely influenced by the presence of proteins and other nutrients in combination with high levels of micronutrients.

Borpuzari *et al.* (2020) found a correlation between the proximate composition of Som and Soalu plants, the primary food plants of Muga silkworm, and the growth parameters of the larvae. In another study, Derara *et al.* (2020) also reported variations in the proximate composition of two cassava leaves that are associated with the production of Eri silkworm cocoons.

Thanga *et al.* (2021) conducted a study to investigate the nutritional properties of various types of castor and tapioca food plants as a food source for Eri silkworms. They found that different food plant varieties had varying proximate compositions, which had an impact on the economic traits of the silkworms. Castor YTP 1 was identified as having high levels of moisture, protein, and total carbohydrate, which resulted in superior economic traits, such as cocoon yield and silk percentage, when the larvae were reared on it. These results suggest that the differences in the biochemical constituents of the leaves could be one of the reasons for variations in the preference and suitability of the leaves as a host plant for Eri silkworms.

Numerous studies have highlighted the importance of various nutrients and biochemical components present in host plants, such as carbohydrates, proteins, fats, leaf moisture, nitrogen content, and secondary metabolites like phenolic compounds and β-sitosterol, for the growth, development, and silk productivity of silkworms. Some of these studies include Hazarika *et al.* (1995); Dutta *et al.* (1997); Singh *et al.* (2000); Bose and Bindroo (2001); Kakati and Kakati (2011), Neog *et al.* (2011). Therefore, a comprehensive analysis of the nutritional properties of various food plants can assist in identifying those that are best suited for silkworm rearing, as suggested by Devi *et al.* (2021).

2.4. Gut digestive enzyme activity

The adequate nourishment of larvae in leaf-feeding insects relies on a balance between the nutritional content of food plants and the activity of digestive enzymes in the digestive tract. According to Applebaum (1985), the digestive system of insects is adapted to the nutritional properties of the host plants. Insects, have mechanisms to digest diverse unbalanced diets (Simpson and Raubenheimer 1993).

Digestion and absorption of food plants are the sources of specific nutrients necessary for normal growth and development in silkworms. Raubenheimer and Simpson (1998) have clarified that insect nutrition is influenced by both behavioral aspects such as selective feeding and physiological processes like digestion, absorption, and allocation. Polyphagous insects rely on a complex interplay of digestive enzymes such as amylase, lipase, cellulase and protease to break down complex food materials into simpler forms in the gut. Proteins are major constituents of herbivorous lepidopteran diets and are broken down into amino acids by proteases. Lipases are produced in the midgut of insects have the ability to decompose different types of dietary lipids, including triacylglycerol and phospholipids, and convert them into fatty acids (Weintraub and Tietz, 1973). Additionally, lepidopteran species that consume diets rich in polysaccharides require the action of amylases and cellulases to break down starch and cellulose, respectively (Valencia-Jimenez *et al.*, 2008; Zibaee *et al.*, 2008; Anand *et al.,* 2010).

The gut serves as the main site for secreting digestive enzymes, processing food, and absorbing nutrients (Pauchet *et al.,* 2008). The digestive system functions to break down and assimilate nutrients essential for maintenance, survival, and reproduction in insects (Oyebanji *et al.,* 2014).

According to Kotkar *et al.* (2009), a balanced action of digestive enzymes is important for obtaining proper nourishment from the diet, which has a significant impact on insect growth and development. The ability of silkworms to secrete digestive enzymes is largely influenced by the nutrient components present in their food, as reported by Manjula *et al.* (2010).

Sarate *et al.* (2012) discovered that the digestive flexibility in polyphagous *H. armigera* larvae is directly influenced by the composition of various diets. Mansouri *et al.* (2013) determined the suitability of host plants for the lepidopteran insect *Phthorimaea operculella* by analyzing larval digestive enzyme activity and nutritional indexes while feeding on two different potato cultivars.

Mardani-Talaee *et al.* (2014) conducted a study on the lepidopteran insect *Chrysodeixis chalcites* and found that the digestive enzyme activity and metabolism of the insect were influenced by the host plants. Their findings suggested that changes in the nutritional content of the food can cause alterations in enzyme activity.

Naseri *et al.* (2014) who studied the digestive enzyme activity and nutritional quality of *H. armigera* fed on various tomato plant varieties demonstrated that they have mechanisms to digest protein-rich plant, reproductive structures and carbohydrate-rich leaves. Furthermore, Oyebanji *et al.* (2014) investigated the digestive enzyme activity in the gut of the polyphagous American cockroach, *Periplaneta americana*, and found that the species' ability to digest a wide range of food substances is due to its feeding habit. These studies demonstrate the significant role of feeding behavior, digestive plasticity, and enzyme activity in determining the nutritional requirements of insects.

Oftadeh *et al*. (2014) conducted a study on how four different varieties of mulberry affected the nutritional indices and digestive proteolytic and amylolytic activities of *Glyphodes pyloalis* Walker. They found that plasticity in food utilization and enzyme activity was important for the consumption rate of nutrients in insects, and was dependent on the host plant cultivars. Enzymes are essential for breaking down complex nutrients into simpler forms that can be absorbed by the body.

Lalfelpuii *et al.* (2014) emphasized that the digestion and assimilation of nutritional materials present in mulberry leaves are crucial to the performance of silkworms. In silkworms, digestive enzymes break down nutrients in the gut into simpler forms which are easily absorbed through the alimentary canal's semi-permeable membrane (Mala and Vijila, 2017).

The nutritional intake and assimilation in silkworms heavily rely on the digestive physiology. Tungjitwitayakul and Tatun (2017) observed that the alpha amylase activity in Eri silkworms varied depending on the rearing diet, whether cassava or artificial. Meanwhile, Khamenei-Tabrizi *et al.* (2020) found that the digestive enzyme activity in *Bombyx mori* also differed depending on the mulberry variety they were fed on. Hence, a comprehensive understanding of both nutrition and the intricate physiology of digestion is fundamental to the Sericulture industry.

2.5. Studies on Gut bacterial symbionts-diversity and functions

The digestive tracts of insects share a common structure, but variations exist in their structure and function based on the insects' feeding behaviours. The gut is composed of three primary regions, including the foregut, midgut, and hindgut, with each region serving distinct roles (Chapman *et al.,* 2013). Douglas (1998), studied on the nutritional interactions between aphids and their gut bacteria where he found that gut bacteria provide essential amino acids to aphids which help them, utilize phloem sap, a diet that is typically lacking in essential amino acids.

Chowdary *et al.* (2002) identified a high mean diversity of gut bacteria in pure Mysore and NB_4D_2 breeds of *B. mori* larvae, with maximum isolates having functional roles in producing amylase, caseinase, gelatinase, lipase and urease enzyme in the gut. Moreover, bacterial mutualists in insect guts help regulate the host's metabolism and protect it from harmful microbes by producing antimicrobial phenolics (Dillon and Charnley, 2002).

Insects have a complex and diverse microbial population in their gut that includes bacteria, fungi, and viruses (Dillon and Dillon, 2004). Broderick *et al.* (2004) investigated the bacterial communities present in the mid-gut of gypsy moth using traditional culture-dependent and culture-independent techniques.

Bacteria are the most abundant and diverse group, with more than 100 identified species. Studies on the symbiotic relationship between aphids and three bacterial lineages have shown that these symbionts provide protection against parasitoid wasps, fungal infections, and heat stress, and can also partially restore the reproduction of aphids (Moran *et al.*, 2005).

According to Rajagopal (2009), the insect gut is estimated to harbour 10 times more microbes than the total number of insect cells, and these microbes possess 100 times more genes than animal genes. He conducted research on the beneficial interactions between insects and gut bacteria, discovering the essential role that bacteria play in the success and establishment of insects. Some bacteria, such as *Wolbachia* and *Cardinium*, were found to manipulate insect populations and sex ratios. Gibson and Hunter (2010) also studied the distribution and functions of endosymbiont microbes in insects.

Engel and Moran (2013) investigated the gut microbiota of insects and found that bacterial diversity varies across species, with social insects such as termites, ants, and bees having distinct and consistent gut communities that play important roles in protection and nutrition. Some symbiotic bacteria also provide benefits to their hosts such as heat tolerance and protection against parasites and pathogens.

The midgut serves as the primary location for food digestion and nutrient absorption in insects. The gut of insects is a complex system where the interaction between microbial and insect enzymes work together to break down the plant components (Shi *et al.*, 2013).

Bhuyan *et al.* (2014) conducted a study to determine the bacterial species composition in the gut of Muga silkworms. They isolated and characterized 12 bacterial species, including both gram-positive and gram-negative bacteria, with *Bacillus sp.*, *Proteus sp.*, and *Escherichia coli* being the dominant groups.

Similarly, Douglas (2015) conducted extensive research on the diversity and functionality of microbial residents of the gut, revealing that resident microorganisms can promote insect fitness by contributing to nutrition, especially by providing essential amino acids, B vitamins, and sterols for fungal partners. Additionally, some microorganisms protect their insect hosts against pathogens, parasitoids, and other parasites by synthesizing specific toxins or modifying the insect immune system.

Ruokolainen *et al.* (2016) showed the association of gut microbiota with the growth of butterfly larvae. The bacterial component of the insect gut microbiota is dominated by four major phyla: Firmicutes, Bacteroides, Proteobacteria, and Actinobacteria (Bibbo *et al.,* 2016). Haloi *et al.* (2016) also found differences in the gut bacteria between healthy and diseased Muga silkworms (*A. assamensis*) through 16S rRNA gene sequencing of bacterial isolates. Some bacterial strains were found in both healthy and diseased silkworms, while others were only present in diseased silkworms.

Gandotra *et al.* (2018b) investigated the bacterial community structure and diversity in the gut of *A. assamensis*, the Muga silkworm. They found that *Bacillus* was the most dominant genera group, followed by *Serratia*, *Pseudomonas*, and *Alcaligens*. They identified several bacterial isolates, such as *Bacillus sp.*, *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Pseudomonas stutzeri*, *Acinetobacter sp.,* and *Alcaligens sp.* inhabiting the gut of Muga silkworms.

Chen *et al.* (2018) conducted a comparative shotgun metagenomic study to explore the diversity and functional roles of gut microbiota in two strains of *B. mori*, namely the inbred strain (P50) and hybrid (QB). In P50 silkworms, 663 bacterial species were identified, while 322 unique species were found in QB silkworms. The bacterial groups *Enterobacter*, *Acinetobacter*, and *Enterococcus* were dominant in both strains.

Similarly, a study by Pandiarajan and Krishnan (2018) compared the gut bacterial diversity of two lepidopteran insects, *Spodoptera litura* and *B. mori*, using both culture-based and culture-independent methods. They found that Enterobacteriacae family was dominant in both insects, with majority of the bacteria belonging to Proteobacteria and Firmicutes phyla. *Enterococcus mundtii* was found to be the most abundant bacteria in both insects.

In addition, the functional annotation revealed the presence of 5x106 protein coding genes in the gut bacterial population of the silkworms. Hou *et al.* (2018) found diverse bacterial species in the intestine of *B. mori*, with dominant phyla including Firmicutes, Cyanobacteria, Proteobacteria, Actinobacteria and Bacteroidetes. The dominant genera were *Enterococcus*, *Staphylococcus*, *Arthrobacter*, *Pseudomonas*, *Lactobacillus*, *Bacteroides*, *Paenibacillus* and *Serratia*, respectively.

According to Adak and Khan (2019), gut microbiota have evolved along with their hosts and have a significant impact on the physical and mental health of an individual. The study highlights that a thorough understanding of the functioning of gut microbiota has led to exciting developments in therapeutics, such as prebiotics, probiotics, drugs, and faecal transplantation, leading to improved health.

González-Serrano *et al.* (2020), highlighted the advantageous roles of gut bacteria in different physiological processes such as nutrition, digestion, and detoxification in *Brithys crini* insects. The authors sequenced the V3-V4 region of the 16S rRNA gene to investigate the composition and diversity of the microbiota in the egg, larval midgut and hindgut, and adult gut of the monophagous moth. They found that the bacterial composition varied across different life stages of the insect.

Similarly,Habineza *et al.* (2019) reported the involvement of gut microbiota in the growth and development of *Rhynchophorus ferrugineus*.

Jing *et al.* (2020) studied the gut bacteria of a type of weevil called *Cryptorhynchus lapathi* and found that Proteobacteria and Bacteroidetes were the most dominant types of bacteria. The gut bacteria were found to be involved in many important functions such as amino acid and vitamin biosynthesis, protein and lipid digestion, energy metabolism, and degradation of plant secondary metabolites.

In another study by MsangoSoko *et al.* (2020a), the gut microbiota of Eri silkworm and White grub (Scarabaeidae) were analysed and found to be diverse with different types of gram positive bacteria present in varying amounts. These gut bacteria may play important roles in the physiology of these insects.

Rajan *et al*. (2020) studied the gut bacteria of *A. mylitta* using a culture-based approach and found several types of bacteria such as *Pseudomonas*, *Erwinia*, *Enterococcus*, *Staphylococcus*, *Bacillus cerus*, *Lactobacillus*, and *Micrococcus* in the gut of the insect. These bacteria may play important roles in the digestion process of the insect.

Wang *et al*. (2020) studied the gut bacteria of *S. litura* larvae reared on different diets and found that the gut bacterial community structure varied significantly depending on the diet. The results suggest that the insect's diet has a close relationship with the composition of its gut bacteria.

MsangoSoko *et al.* (2021) investigated the bacterial composition and diversity in the gut of Eri silkworms through a culture-dependent and metagenomic approach. They found a diverse community of both cultivable and non-cultivable gut bacteria with cellulolytic, lipolytic, and nitrate reductase activities, indicating their potential involvement in food digestion and nutritional supply to the host. The bacterial isolates could serve as a source for discovering novel genes and biomolecules for biotechnological applications.

Devi *et al.* (2022) used 16S rRNA gene sequencing to compare the gut microbiota of healthy and Tiger band disease-infected Oak Tasar silkworms (*A. proylei*). They found that the bacterial composition of the midgut was different between the two groups, with different abundant bacterial genera.

Zhang *et al.* (2022) emphasized the importance of the intestinal microbiota in maintaining the health and nutrition of the silkworm, and highlighted the need for further research to better understand how changes in the gut microflora in diseased condition may impact silkworm health and productivity.

Xue *et al.* (2023) studied the bacterial community of *Apolygus lucorum*, an omnivorous pest insect, throughout its entire life cycle. They found that Proteobacteria, Firmicutes, and Actinobacteria were the most abundant bacterial phyla in all developmental stages. *Erwinia* and *Lactococcus* were the most prevalent genera

In a recent study, Gogoi *et al.* (2023a) analysed the seasonal variation of the gut microbiota of Eri silkworms using 16S rRNA gene sequencing. They found three phyla, 13 families, and 22 genera in the gut microbiota across the holometabolous life cycle during summer and winter seasons. The summer gut isolates showed high diversity and were dominated by the Bacillus genus, which had lignocellulose activity. Their findings suggest that the gut bacterial microbiota of *S. ricini* is affected by the holometabolous life cycle and varies across seasons.

2.6. Functional roles of gut bacteria

2.6.1. Growth and development

The microorganisms present in the gut can establish various associations with their hosts, including nutritional commensalism or symbiosis, according to research by Colman *et al.* (2012). Insects such as termites have gut bacteria that can fix dinitrogen into ammonia and synthesize vitamins and amino acids, which are required for their development (Borm *et al.*, 2002; Frohlich *et al.,* 2007; Kneip *et al.*, 2007; Brune, 2014; Brune and Dietrich, 2015).

Ruokolainen *et al*. (2016) demonstrated that gut microbiota is associated with the growth rate of *Glanville fritillary* butterfly, with 50% of the variation in larval growth rate attributed to the gut microbial composition.

Similarly, Habineza *et al.* (2019) found that gut bacteria play a crucial role in promoting the growth and development of Red palm weevil (RPW) by regulating its nutrient metabolism. The two dominant gut bacteria, *Lactococcus lactis* and *Enterobacter cloacae*, were also found to increase the protein content of RPW.

Additionally, Yeruva *et al.* (2020) reported the beneficial role of gut bacteria in nutrient absorption, which is related to the growth, development, and economic traits of silkworms.

2.6.2. Resistance against pathogens and providing immunity

Insects have developed various strategies such as antimicrobial peptides and chemical compounds to combat pathogens and parasites and provide immunity to the host (Sackton *et al.,* 2007). In the gypsy moth, *E. faecalis* is capable of acidifying its surrounding environment, which allows it to colonize alkaline niches and protect the gut against pathogenic toxins that are activated in alkaline conditions, such as those produced by *B. thuringiensis* (Broderick *et al.,* 2004).

Moran *et al.* (2005) have studied three bacterial lineages that are symbiotic in aphids and found that the symbionts play a role in protecting the aphids against parasitoid wasps, fungal infections, and heat stress, and also improve their reproductive capabilities.

Sun *et al.* (2016) observed changes in the composition of the gut bacterial population in silkworms infected with BmCPV and suggested that these changes may be related to the immune response of the host. In addition, *Enterococcus sp.* and *Staphylococcus sp.* found in *B. mori* have been shown to play a role in the immune response against infections (Sun *et al.,* 2017).

Shin *et al.* (2017) isolated a Mycobacterium strain from the gut of another burying beetle, *N. concolor*, which produced novel chlorinated cyclic peptides, nicrophorusamides A and B. Among these two antibacterial peptides, nicrophorusamide A showed high antimicrobial activity against several human pathogens such as *S. aureus* and *Enterococcus faecalis*.

Shao *et al.* (2017) discovered that the gut of the cotton leafworm, *S. littoralis*, contains symbiotic *E. mundtii,* which produces an antimicrobial peptide called mundticin that inhibits human-pathogenic and entomopathogenic enterococci such as *E. faecalis* and *E. casseliflavus*.

Recent studies have focused on identifying specific gut bacteria that exhibit antimicrobial activity. Liu *et al.* (2018) discovered *Bacillus pumilus* in the gut of *B. mori,* which exhibited antiviral activity against the nucleopolyhedrovirus of this insect.

Heise *et al.* (2019) isolated a gut symbiont, *Serratia marcescens*, from the burying beetle *Nicrophorus vespilloides*, which produced an antibacterial cyclic lipopeptide known as serrawettin W2. This lipopeptide demonstrated strong antimicrobial activity against human pathogens, including MRSA and *Listeria monocytogenes*.

Chevrette *et al.* (2019) conducted a comprehensive survey of diverse insects from 15 orders and found that Streptomyces associated with insects have a stronger ability to inhibit antimicrobial-resistant bacteria compared to soil-derived Streptomyces.

Some gut bacteria have been found to produce antimicrobial compounds that could be used to treat multidrug-resistant human pathogens (Jang and Kikuchi, 2020). Zhang *et al.* (2022) discovered that *E. faecalis* LX10 provides immunity to silkworms against *Nosema bombycis* infection. Another recent study found that *Enterococcus hirae* SX2, isolated from the midgut of Eri silkworms, has antimicrobial activity against insect pathogenic bacteria (Unban *et al*., 2022).

2.6.3. Detoxifications

Gut bacteria living in symbiosis with their host have an important role in neutralizing toxic substances and allelochemicals produced by plants. Genta *et al.* (2006) reported the role of midgut microflora in degrading plant glucoside salicin. Xia *et al.* (2017) identified the detoxifying role of abundant bacteria, including *Enterobacter cloacae*, *Enterobacter asburiae*, and *Carnobacterium maltaromaticum*, in the Diamondback moth (*Plutella xylostella*).

According to Chen *et al.* (2020), Stenotrophomonas bacteria were found to enhance the host's resistance to chlorpyrifos, an organophosphate insecticide. They found that essential amino acid levels were higher in the guts of silkworm larvae monoassociated with *Stenotrophomonas*, indicating a highly species-specific pattern. This nutrient provisioning boosted host fitness and enabled larvae to more efficiently cope with the harmful effects of toxic chemicals.

2.6.4. Host metabolism

Tayasu *et al.* (1994) established that gut bacteria play a crucial role in insect host's nitrogen metabolism, and they are responsible for fixing atmospheric nitrogen in fruit flies and termites. Yatsunenko *et al.* (2012) found that the gut microbiota is responsible for the regulation of vitamin synthesis in the host. Yano *et al.* (2015) and Neuman *et al.* (2015) discovered that the gut microbiota regulates the synthesis of neurotransmitters and intestinal endocrine hormones in the host, respectively.

Liang *et al.* (2018) isolated bacterial *E. mundtii* from the gut of *B. mori*, which was found to produce essential metabolites required for host growth and development. Rajan *et al.* (2020) found that *Turicibacter sp.* produces lactate in the gut. Li *et al.* (2022c) discovered that *B. subtilis*, a gut bacterium in silkworms, produces B vitamins that enhance the host's health status. Liang *et al.* (2022) reported that *E. casseliflavus* is involved in the metabolic process of silkworms by producing L-Tryptophane that can modulate the host's physiology.

2.6.5. Digestive enzymes produced by gut bacteria

The gut plays a significant role in the secretion of digestive enzymes, nutrient absorption, and food digestion. The microbial community present in the gut produces enzymes that aid in the breakdown of carbohydrates, such as cellulose, pectin, xylan, and starch, and hydrolysis of lipids and other food components of the host. This process contributes to the provision of key nutrients required by the host (Pauchet *et al.,* 2008; Gao *et al.*, 2007; Feng *et al*., 2011; Gandotra *et al*., 2018b).

Fukatsu and Hosokawa (2002) found that loss of microorganisms can lead to abnormal development and reduced survival of insect hosts. Dillon and Dillon (2004) reported that digestive enzyme-producing bacteria in the midguts of Lepidopteran insects can aid in the digestion of mulberry leaf constituents such as cellulose, xylan, pectin, and starch.

Genta *et al.* (2006) found that the midgut of *Tenebrio molitor* larvae treated with antibiotics to create sterile conditions produced amylase, cellulase, and β-glucosidase. They suggested that microbial enzymes may have a non-essential role in digestion.

Park *et al.* (2007) used culture-dependent methods to identify xylynase and pectinase producing gut bacteria in nine beetle species, mainly belonging to the gamma proteobacteria group. Their results suggested that these bacteria may play a crucial role in the digestion of woody diets in insects.

In a study conducted by Anand *et al.* (2010), they investigated the gut bacteria of the mulberry silkworm, *B. mori*, and their ability to produce digestive enzymes. The focus was on the screening of polyssacharide degrading enzymes such as amylase, cellulase, pectinase, and xylanase. Three bacterial isolates, *P. vulgaris*, *K. pneumoniae*, and *C. freundii*, were found to have cellulolytic and xylanolytic activity, while two other isolates, *P. fluorescens* and *Erwinia sp*., showed pectinolytic activity and *K. pneumoniae* was able to produce amylase. *Aeromonas sp.* was identified as having cellulose and xylanolytic activity, while *S. liquefaciens* utilized three polysaccharides, including cellulose, xylan, and pectin. *B. circulans* showed activity for all four enzymes with varying effectiveness. Overall, the study revealed that gut bacteria work together synergistically to produce digestive enzymes, which contribute to the growth of larvae.

Feng *et al.* (2011) isolated and screened 56 gut bacteria strains from *B. mori* larvae fed on different forage and found lipase activity in nine isolates from six genera. Matteotti *et al.* (2011) studied glucosidase activities by functionally screening a genomic DNA library from the gut microbiota of termite and reported the presence of gut bacterial colonies that produce glucosidase enzyme activity.

Khyade and Marathe (2012) investigated the diversity of gut bacteria in the mid gut of the fifth instar larvae of silk worm *B. mori* (RACE: PM X CSR2) and found that these larvae consume mulberry leaves which are rich in carbohydrates such as pectin, xylan, cellulose and starch. Some of the digestive enzymes responsible for breaking down these carbohydrates might be produced by the mid gut bacteria. The researchers isolated eleven bacterial strains from the digestive tract of multivoltine, cross breed silk worm, *B. mori* (PM x CSR2), which included *B. circulans* (Gram positive) and *Proteus vulgaris, Klebsiella pneumonia*, *Escherichia coli, Cittrobacter freundii*, *S. liquefaciens*, *Enterobactor sp.*, *Pseudomonas fluorescens*, *P. aeruginosa*, *Aeromonas sp*. and *Erwinia species* (Gram negative). Among these, three isolates – *P. vulgaris*, *K. pneuomonae* and *C. freundii* were cellulolytic and xylanolytic, while *P. fluorescens* and *Erwinia sp.* were pectinolytic, and *K. pneuomniae* was found to degrade starch. *Aeromonas sp.* was able to utilize Carboxymethyl (CM) cellulose and xylan, while *S. liquefaciens* was able to utilize CM cellulose, xylan and pectin. *B. circulans* was able to utilize all four polysaccharides with varying degrees of efficiency. The researchers also noted an increase in cellulolytic bacteria with each instar of the larvae.

Prasanna *et al.* (2014) conducted a study to characterize *Bacillus megaterium*, which is an amylase-producing bacterium found in the gut microbiota of silkworms (*B. mori*). The researchers used phenotypic and biochemical tests, as well as 16S rRNA gene sequencing, to confirm the identity of *B. megaterium* and found that the bacterium produces α-amylase enzymes that work together with the host's endogenous digestive enzymes (such as amylases, cellulases, pectinases, xylanases, etc.) to digest starch compounds.

Liang *et al.* (2015) investigated the ability of silkworm larvae to produce digestive enzymes in a Bio-regenerative Life Support System (BLSS) and Traditional Rearing Method (TRW). They identified and compared the amylase and cellulose producing gut bacteria in groups, and found *Alternaria sp.*, *Preussia sp.*, and *Coprinellus radians* in TRW, and *Enterococcus*, *Erwinia*, and *Pantoea* in BLSS. These bacteria were able to produce cellulase and amylase, and the researchers suggested that utilizing dominant populations with enzymatic activity could enhance gut microecology, as well as improve yield and protein quality.

Dantur *et al.* (2015) isolated 118 bacterial strains from the intestine of *Diatraea saccharalis* larvae and tested them for cellulolytic activity using Carboxymethyl Cellulose (CMC) as a substrate. *Bacillus* and *Klebsiella* showed the highest activity among the strains tested. These findings highlight the potential role of gut bacteria in insect digestion and may have applications in biotechnology.

Gandotra *et al.* (2018a) conducted a study on the gut bacterial isolates of three lepidopteran insects, namely *A. assamensis*, *H. armigera*, and *P. xylostella*, to identify their enzyme-producing capabilities. They found that *A. assamensis* had the highest number of isolates with cellulase, lipase, amylase, and gelatinase activities, while *H. armigera* had a moderate number of isolates with these activities, and *P. xylostella* had a relatively low number of isolates with these activities. Bhuyan *et al.* (2018) also found a cellulose enzyme-producing strain of *B. pumilus* in the midgut of muga silkworm *A. assamesis*. They suggested that gut microflora can play a significant role in digestion and disease resistance and can be used as a potential probiotic component to improve silk productivity.

Pandiarajan and Revathi (2020) conducted a study to identify cellulolytic bacterial species present in the gut of silkworm *B. mori* by conducting a screening test on CMC agar medium. Their research found that *Bacillus aryabhattai* and *Bacillus sp.*

exhibited the highest cellulolytic activity, based on the zone of clearance, among other species. Their findings suggest that the cellulase produced by the gut microbiome can serve as a useful biocatalyst in the field of biofuels recovery and can also be utilized as a Phytobezoar for the treatment of cattle and humans.

Maske *et al.* (2021) focused on the significance of *lactobacilli* that produce digestive enzymes for human health. They noted that *lactobacilli* play an important role in producing lactase, proteases, peptidases, fructanases, amylases, bile salt hydrolases, phytases, and esterases.

Similarly, MsangoSoko *et al.* (2021) found cellulolytic enzyme activity in bacterial isolates belonging to *Bacillus sp.* (88%), *Pseudomonas sp.* (8%), and *Enterococcus sp.* (4%) in Eri silkworm (*S. ricini*) bacterial isolates. Gogoi *et al*. (2023a) identified 43 isolates with cellulolytic and 29 isolates with xylanolytic potential in the gut of *S. ricini*.

In another study by Danu *et al.* (2023), they investigated the cellulolytic potential of gut bacterial isolates from four white grub species found in the Indian Himalayas. They characterized 178 bacterial strains and identified 95 gut bacterial isolates with cellulolytic activity from all four species. Their findings showed that *B. stratosphericus*, *B. cereus*, *Bacillus sp.*, and *Paenibacillus ginsengagri* strain were the most potent cellulose-degrading bacteria isolated from the gut of *B. coriacea*, *H. longipennis*, *H. setticollis*, and *A. bengalensis*, respectively. The study emphasized the significant role of gut bacteria in host organisms' digestive mechanism, which ultimately contributes to their overall health.

2.7. Factors affecting the bacterial diversity of gut

Insects can harbour gut microbial communities ranging from simple to complex community (Lilburn *et al*., 2001; Handelsman *et al.,* 2005; Vasanthakumar *et al*., 2006). According to Dillon and Dillon (2004), the structure, type of food ingested, gut pH, and redox potential are significant factors that play a role in selecting and enriching the bacterial species.

Studies have shown that the gut microbiota of silkworms is mainly composed of bacteria from the phyla Firmicutes, Proteobacteria, and Actinobacteria, but the composition and abundance of gut bacteria in silkworms can vary significantly depending on several factors, such as the host genotype, gut structure, diet, developmental stage, and environmental factors, including host phylogeny (Yun *et al*., 2014). Kim *et al*. (2017) also demonstrated that the type of diet, developmental stage, and gut compartment are important factors in shaping gut bacterial communities in two *Cerambycidae* species.

2.7.1. Host Genotype

Studies have revealed that the gut microbiota of silkworms can be influenced by host genotype and phylogeny. In particular, different individual silkworm strains from species have been found to have unique gut bacterial profiles, which could affect their health and susceptibility to diseases (Gandotra *et al.,* 2018b). Similarly, Wang *et al.* (2020) reported differences in bacterial communities between *A. pernyi* and *B. mori*.

2.7.2. Gut pH

The pH of the gut contents is a crucial factor that affects insect digestion, as it is necessary for the activities of digestive enzymes. Bacteria typically grow optimally at a pH range of 6-7, and there is a strong correlation between the pH optima of digestive enzymes and the gut pH. Therefore, when estimating enzyme quantities, it is essential to take into account the physiological pH of the intestinal tract, which is typically between (Terra and Ferreira, 1994; Regel *et al*., 1998; Funke *et al*., 2008).

2.7.3. Developmental stage

The life stages of insects can vary between species and play a crucial role in their development. Age is also an important factor that can affect the diversity and evenness of gut bacteria in silkworms. Gonzalez-Serrano *et al.* (2020) reported that gut bacteria are specific to certain developmental stages and found variations in gut bacterial diversity in the larval and adult stages of the *Brithys crini* moth. These results suggest that developmental stage is a significant factor affecting the composition and potential functions of the gut microbiome. Research has shown that the gut bacterial communities of silkworms change significantly during their growth and development stages. For example, as larvae mature, the gut bacterial diversity tends to increase (Chen *et al.,* 2018; Tan *et al*., 2022). Similarly, Xue *et al.* (2023) discovered that microbial community composition and relative abundance shift dynamically at different life stages, indicating that different bacterial phyla and genera may play specific roles in metabolism, nutrition absorption, detoxification, and reproduction.

2.7.4. Host's gender

The gender of silkworms has been shown to affect their gut microbiota composition. Research has demonstrated that male and female silkworms may have different gut microbiota compositions (Sun *et al.,* 2016).

2.7.5. Chemical factors

The composition of gut bacterial diversity in insects can be influenced by the use of insecticides and disinfectants, which can in turn affect the host's physiology. Studies have shown that the use of insect growth regulators and antibiotics can also alter the diversity of gut bacteria. Moreover, disrupting the community structure of gut microbes through these perturbations can have adverse effects on the nutrition of insects, as microbial symbionts play important roles in complementing their host's nutrition (Bracke *et al*., 1978; Kaufman *et al*., 1989; Kaufman and Klug, 1991; Li *et al*., 2020; Lin *et al*., 2020; Lu *et al*., 2022).

2.7.6. Environmental Factors

The insect gut is colonized by a wide mixture of microorganisms as a result of its continuous contact to the external environment (Steinhaus, 1960). Studies also suggested that poor environmental conditions can lead to the proliferation of harmful bacteria and the suppression of beneficial ones, resulting in infection or disease that in turn affect the overall health and reduced silk production. The seasonal factor and environmental factors, such as temperature, humidity and infection, can also influence the diversity of gut bacteria in silkworms (Haloi *et al*., 2016; Sun *et al*., 2017; Kumar *et al*., 2019; Sun *et al*., 2022; Gogoi *et al*., 2023a).

2.7.7. Diet of host organism

The influence of factors on the gut microbial community of insects is complex and interdependent. Studies have demonstrated that the diet and taxonomic identity of the host have a significant impact on the composition of the gut microbial community. Diet, in particular, has been found to be a crucial determinant of gut bacterial diversity in silkworms. Feeding on different types of host plants, artificial or antibiotics-treated diets, or diets that differ from their natural food sources, can alter the gut bacterial composition and function, which may ultimately affect the physiology and silk production of the host. (Broderick *et al.,* 2004; Colman *et al.,* 2012; Liang *et al.*, 2014; Hao *et al.,* 2019; Li *et al.,* 2022c).

2.8. Role of diet on gut bacterial diversity, community structure and functioning

Insects exhibit a wide range of food preferences, with some being polyphagous, which means their food choices vary greatly. The type of food or diet consumed by the host insect has been identified as a crucial factor that significantly affects the structure and functions of the gut bacterial community. Numerous studies *viz.,* Appel and Maines, 1995; Turnbaugh *et al.*, 2009; Scott *et al.*, 2013; Ringo *et al.*, 2016; Shukla *et al.*, 2016; Rimoldi *et al.,* 2019; Panteli *et al.*, 2021; Nova *et al.*, 2022 have investigated the influence of dietary factors and host plants on the gut microbial population and structure of insects, providing valuable insights into their relationships.

Broderick *et al.* (2004) conducted a study on the effects of different diets on the microbial composition in larvae gut using both culture-dependent and culture-independent methods.They observed significant differences in the microbial communities among larvae feeding on a sterilized artificial diet, aspen, larch, white oak and willow.

Studies on Lepidoptera have also supported the idea that a more diverse diet leads to a more diverse larval midgut microbiome. It is therefore predicted that limitations in food availability may decrease microbiome diversity, which could result in the organism investing less in immune mechanisms for microbiota control and in the expression of AMP genes, a factor that is often overlooked in eco-immunological research (Broderick *et al.,* 2004; Xiang *et al.,* 2006; Pinto-Tomás *et al.,* 2011; Priya *et al.,* 2012; Mason and Raffa, 2014).

Several studies have found a positive correlation between diet diversity and microbiome diversity, which may make the microbiome more adaptable to changes. Diet diversity has been found to be positively correlated with microbiome diversity, which can increase the adaptability of the microbiome to perturbations (Bolnick *et al.,* 2014).

The insect gut is colonized by microorganisms through food, and these microorganisms play an important role in digestion and metabolism. Although most of these gut microbes are commensals or parasites, some are known to be beneficial for their hosts (Krishnan *et al.,* 2014).

Liang *et al.* (2014) found that the gut bacteria of silkworms in the Bio-Regenerative life support system Rearing Way (BRW) decreased significantly compared to that of the Traditional rearing Way (TRW), contributing to the decrease of silkworm physiological activity. These changes in the gut microbial composition of the silkworms affect the balance between bacterial growth and nutrient processing in the gut, which is maintained by immune mechanisms like generation of reactive oxygen species and antimicrobial peptides. The composition of gut microbiomes is therefore largely influenced by the host's diet.

Perez-Cobas *et al.* (2015) found that the gut microbiota of a lab-reared omnivorous insect changed depending on its diet, suggesting that the insect's varied diets contribute to the flexibility of its gut microbiota. Bibbo *et al.* (2016) found that the gut bacterial composition differs depending on the type of diet, such as a western diet or a fiber-rich diet. A specific diet can promote the growth of certain bacterial strains, altering the host's fermentative metabolism, and affecting intestinal pH, which may lead to the development of pathogenic bacteria. A high-fat diet can also lead to the development of a pro-inflammatory gut microbiota, increasing intestinal permeability and circulating levels of lipopolysaccharides.

According to Krams *et al.* (2017), the diversity of diet has a significant impact on both the diversity of the microbiome and the expression of antimicrobial peptide (AMP) genes in the greater wax moth (*Galleria mellonella*), which in turn affects the organism's immune response. The researchers found that the expression of immunity-related genes, such as Gloverin and Galiomicin, increased with greater dietary diversity, which may have acted as a prophylactic defense against opportunistic infections and helped to regulate the gut symbionts. However, they also noted that this diverse diet resulted in a higher cost to the organism's immunity.

Similarly, diet-induced changes in gut microbial composition have been observed in humans (Shin *et al.,* 2017), with specific food consumption leading to predictable shifts in bacterial genera that affect host immune and metabolic parameters. Chen *et al.* (2018) found variations in gut microbial composition and their associated functions in domesticated mulberry silkworms and their wild relatives, which can have a significant impact on host physiology.

Dong *et al.* (2018) found that there were significant changes in the composition and diversity of gut microbiota. The abundance of dominant bacterial groups such as Cyanobacteria, Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria varied between silkworm strains that were reared on different diets. Furthermore, the diversity of gut microbiota was lower in the silkworm strains that were reared on the artificial diet, which may have affected the host's nutrient metabolism and immune resistance these changes could be due to the long evolutionary history of silkworms feeding on mulberry leaves. Scully *et al.* (2018) found that host plants play a crucial role in shaping the gut microbiota of lepidopteran insects. Zhang *et al.* (2018) also found a correlation between the gut microbial composition and the host's diet.

Jones *et al.* (2019) found that host plant is a major driver in shaping the gut microbiota of two polyphagous insects, fall armyworm (*S. frugiperda*) and corn earworm (*Helicoverpa zea*). However, the variation in microbiomes could also be attributed to differences in insect physiology, gut region, and local factors.

Colman *et al.* (2012) provided compelling evidence that supports the impact of the host's diet and taxonomy on the composition of the gut microbiota in insects, based on their analysis of 16S rRNA gene sequencing data, when the diet of *B. mori* was changed from mulberry leaves to an artificial diet.

Huang *et al.* (2021) used a high-throughput 16S rRNA sequencing platform to investigate the impact of diet on the gut microbiome of nine bamboo-feeding insects from different insect orders. They observed variations in bacterial composition among the different dietary niches of bamboo leaf, shoot, and sap. *Trichoplusia ni* is a polyphagous insect that feeds on a variety of crops.

Leite Mondin *et al.* (2021) proposed that insects obtain their gut microbiota from their diet, and may acquire phytochemical-degrading gut bacteria to adapt to the available diet. On the other hand, Luo *et al.* (2021) investigated the impact of an abnormal diet on the gut microbiota of bugs, and their findings indicated that variations in gut microbiota caused by a diet change may be harmful to the insect host.

Yuan *et al.* (2021) examined the gut bacterial community structure of the lepidopteran insect *Grapholita molesta* reared on different host plants, and their results using high-throughput sequencing analysis revealed that the host's diet played a significant role in shaping the gut bacterial composition, which in turn contributes to the adaptation mechanism of the host insect.

Polyphagous insects have the ability to consume a variety of plant combinations which has a significant effect on their physiology. Han *et al.* (2023) used high-throughput sequencing to investigate the effect of different host plants on the gut bacterial communities of *S. frugiperda*. The study confirmed that the diversity and structure of gut bacteria of *S. frugiperda* can be significantly influenced by the host plant. Chen *et al.* (2023) recently reported significant changes in gut bacterial diversity and abundance at different taxonomic levels in *B. mori* fed on cadmium-polluted mulberry leaves.

2.9. Diversity analysis of gut microbial population

Several diversity indices can be used to analyze microbial community diversity, including the Simpson index, Shannon-Wiener index, Berger-Parker index, Fisher's alpha index, and Pielou's evenness index (Magurran, 2005; Studeny *et al.,* 2011; Dong *et al.,* 2018). To fully analyse microbial diversity within a community, both species richness and evenness must be considered, which offers a deeper understanding of community structure (Begon *et al.,* 2006). To quantify and describe the numerical structure of diversity within a community, diversity indices can be used, which provide a better understanding of the relative abundance and species richness of the microbes (Rani *et al.,* 2009). Studies have shown that species diversity within natural communities is never even (Studeny *et al.,* 2011).

Zouache *et al.* (2011) used the Shannon-Wiener and Simpson indices to analyze the bacterial diversity of *Aedes aegypti* and *Aedes albopictus* (field-caught mosquitoes) collected from distinct geographical areas of Madagascar.

In a study on Muga silkworm, Gandotra *et al.* (2018b) used several diversity indices, including the Shannon-Wiener index and Simpson index, to analyse gut bacterial isolates collected from different locations in North East India. The results showed significant differences in the Shannon and Simpson diversity indices of gut bacteria from *A. assamensis* collected from different regions. MsangoSoko *et al.* (2020b) also used diversity indices, including the Shannon index and Chao1 and observed species metrics, to determine gut microbial diversity within their samples.

Xue *et al.* (2023) used several diversity indices, including the Chao1, ACE, Shannon, Simpson, and Good's coverage indices, to analyse the gut bacterial diversity of *Apolygus lucorum* at different developmental stages. They used the Kruskal-Wallis test to analyse the differences in the relative abundance of species within three or more groups.

2.10. Bioinformatics and Statistical analysis

In addition to diversity indices several biostatistics analyses, such as Principal Coordinate Analysis (PCoA), Statistical analysis of metagenomic profile (STAMP) Heatmap analysis, Co-occurrence analysis, Venn diagram analysis, and PICRUSt data analysis have provided a better understanding of gut bacterial metagenomic and functional profile as well as their interactions, relationships with their respective host organism (Parks and Beiko, 2010; Sun *et al.,* 2016; Dong *et al.,* 2018; Chen *et al.,* 2018; Yeruva *et al.,* 2020; Wang *et al.,* 2020).

Therefore, from the above review of literature study we have identified the importance aspect of gut bacteria and their influential roles on host organism and their important transmission depending on certain factor. Based on these factors, the following aim and objectives were constructed for the present study:

Aim of the study- The main aim of this research is to comprehensively unravel the complex interrelationship between the gut bacterial composition of *Samia ricini* silkworms and their host plants, as well as their respective functional contributions to host physiology.

Objectives of the study

1. To study the diversity of bacterial communities in the larval gut of silkworm *Samia ricini* feeding on different food plants using a culture independent method.
2. To isolate and characterize some beneficial gut bacteria using a culture dependent method.
3. To study some nutritionally important cultivable gut bacteria through quantitative enzymatic assay.