Chapter - I

REVIEW OF LITERATURE

1.1 Global, National and State scenario of sericulture

Sericulture is an agro-based industry including three main components: i) cultivation of host plant, ii) silkworm rearing and iii) reeling and spinning of silk. India is second highest producer and also consumer of the silk in the world followed by china (Vijayaprakash and Dandin, 2005) (Figure 1.1). There are four varieties of serigenous insects (silk producing insects) reared in India viz. Mulberry, Tasar, Muga and Eri silkworm. North eastern part of India is the only region in the world where all four varieties of silkworm are reared (Figure 1.2). About 14% raw silk of global production is operated in India. Among them, more than 60% of total silk production is mulberry silk. Mulberry sericulture is practiced throughout the India. However, it is practiced in mainly five states of India viz. Karnataka, West Bengal, Andhra Pradesh, Tamil Nadu and Jammu & Kashmir. These five states collectively produced 98% raw silk of total production in the country. India produced almost 20,000 MT raw silks whereas the demand was 26,000-28,000 in the country.

Sericulture of West Bengal is dominated by mulberry culture. On the average 84% of total silk production in West Bengal is produced by mulberry culture. Tasar culture is practiced in few districts of West Bengal only and about 2% of state silk production is contributed by Tasar silk cultivation.

According to Mukherjee (1992), seven major silk producing regions are found in West Bengal (Figure 1.3). These seven regions were classified as:

1. Ganga-Mahananda Doab- Malda district
2. Murshidabad district
3. Nalhati-Bolpur Region- Birbhum district
4. North and South Dinajpur
5. Darjeeling district
6. Purulia-Bankura
7. Terai and Duars- Jalpaiguri, Alipurduar and Coohbihar district

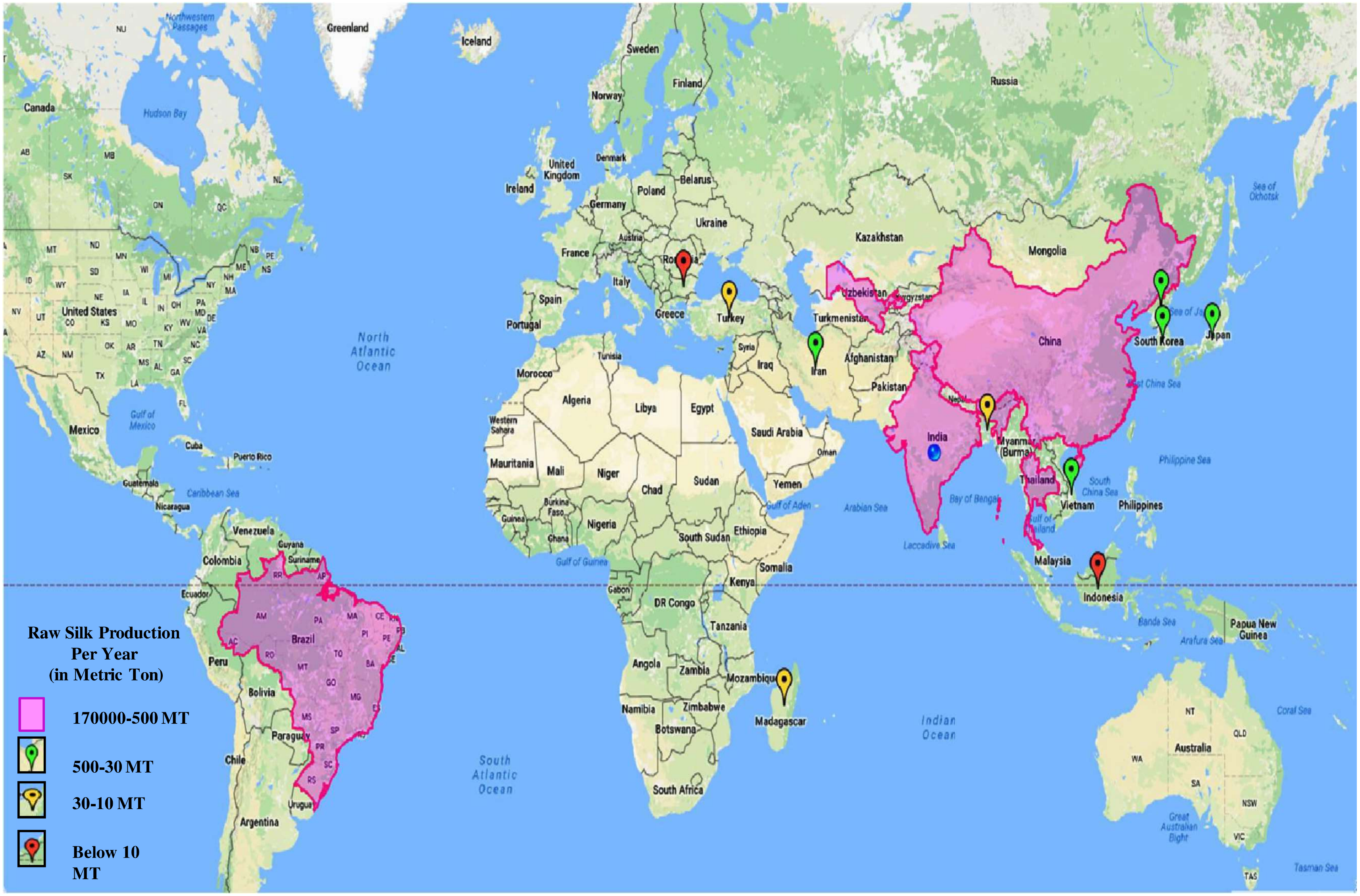

Figure 1.1: Global scenario of sericulture

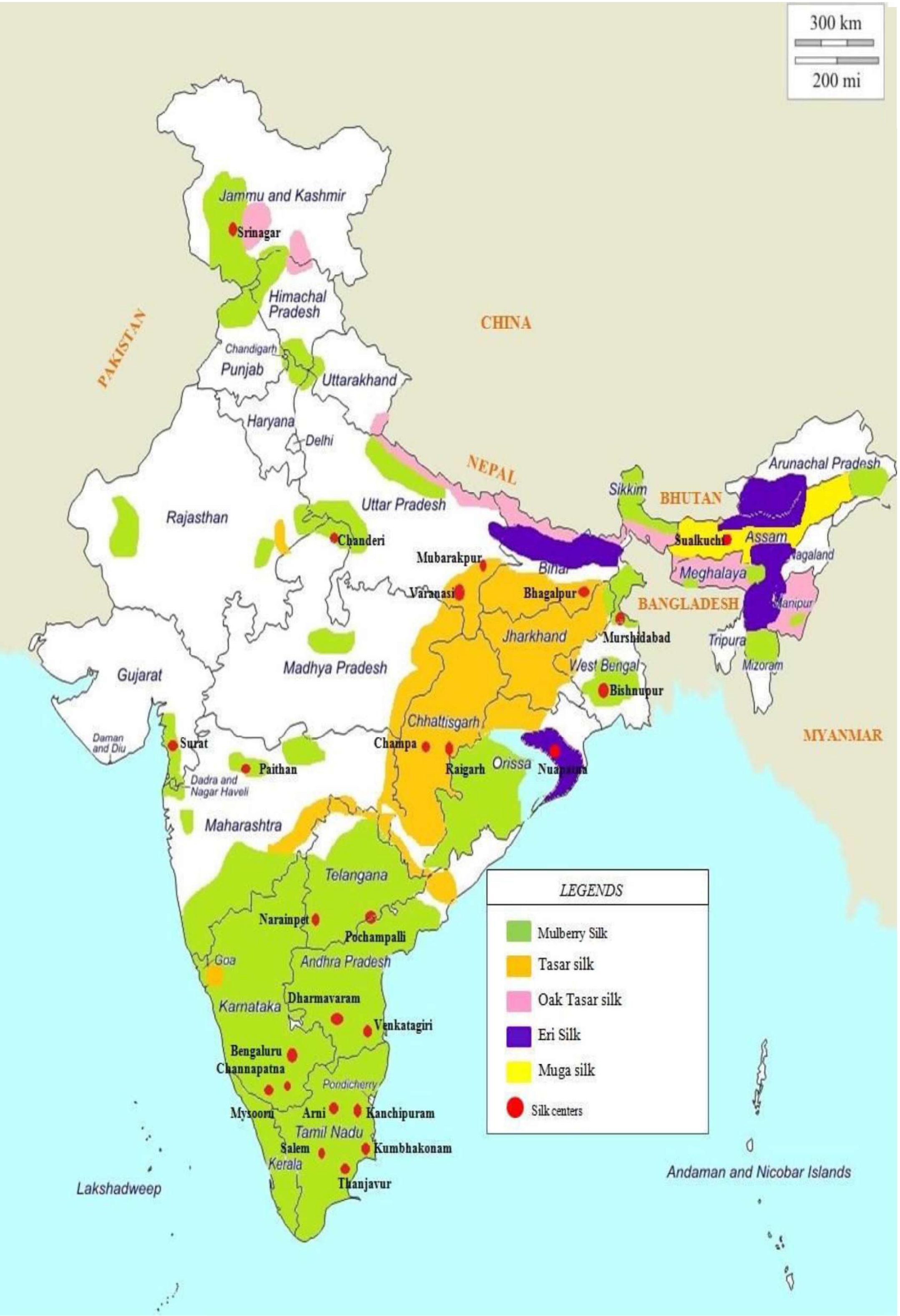

Figure 1.2: Major silk producing regions in India

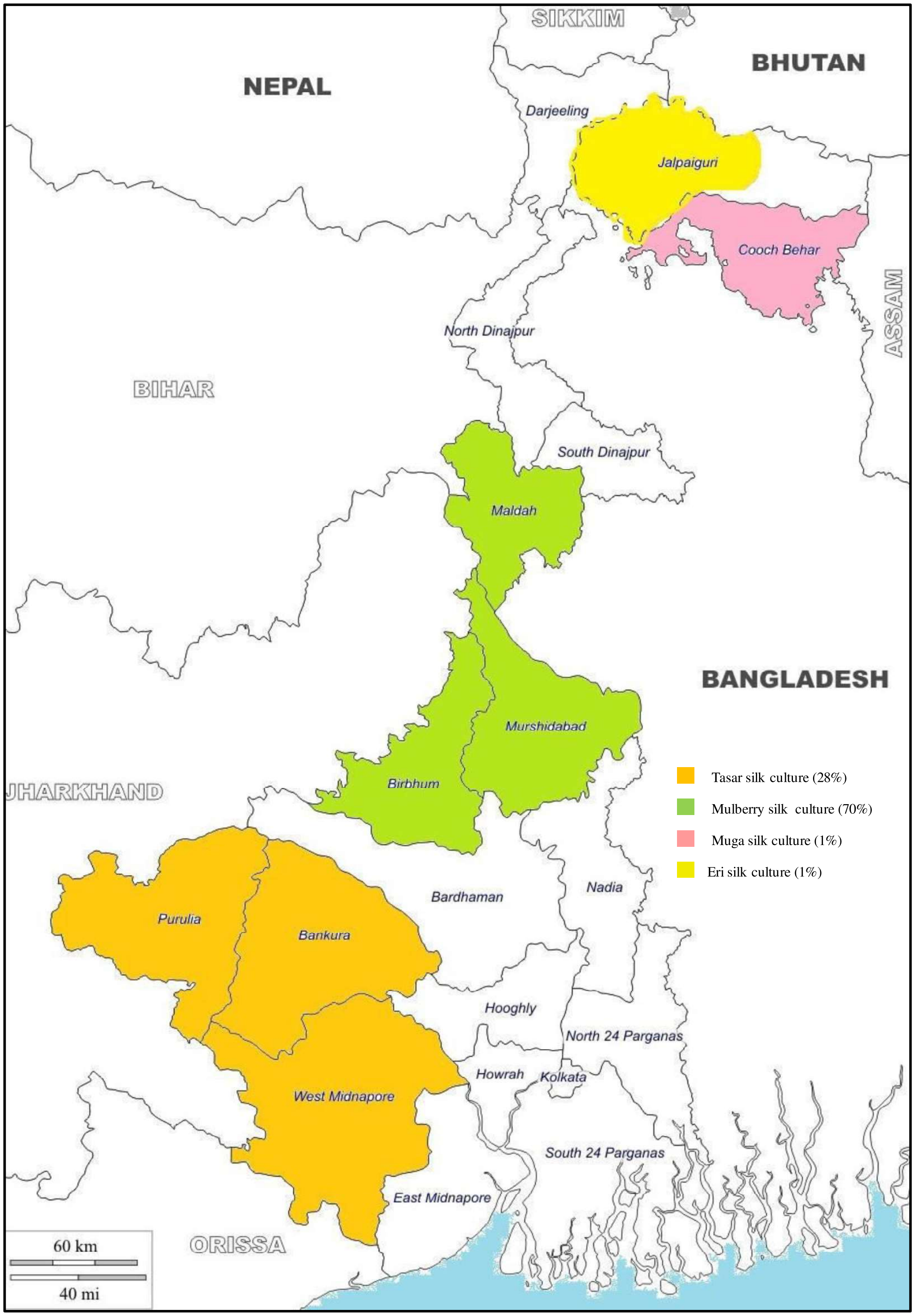

Figure 1.3: Major silk producing regions in West Bengal

Though, West Bengal was a pioneer state in Indian sericulture, but it took third position in mulberry silk production. In West Bengal, mulberry cultivation performed in 2000 villages with almost 37,883 acres plantation area. Malda, Murshidabad and Birbhum districts have been considered as major silk producing area in West Bengal. Bulk amount of silk produced in these three districts. Additionally, Murshidabad is famous for silk weaving. Silk weaving specially mulberry silk also is done in Bishnupur and Bankura district. Malda district contributes 74% of total silk production of the state and takes up leading rank in West Bengal for mulberry silk cultivation (Roy and Roy Mukherjee, 2015).

1.2 Mulberry silk culture

In India mulberry silk culture was performed by moriculture (mulberry plant culture) and silkworm rearing. There are about 1000 varieties of silkworm (Barnet, 1963), among them bivoltine and multivoltine races are particularly used for rearing in India. Silkworm, *Bombyx mori* L. is mostly domesticated and is believed to have derived from the original mandarina silkworm *Bombyx mandarina* Moore (Krishnaswami *et al.,* 1973). *Bombyx mori* L. is a monophagous insect which has special significance in sericulture industry.

The taxonomic position of silkworm according to scientific classification is as follows:

Kingdom: Animalia
Phylum: Arthopoda
Class: Insecta
Order: Lepidoptera
Family: Bombycidae
Genus: Bombyx
Species: *B. mori*
Binomial name: *Bombyx mori* L.

Silkworm totally depends on the mulberry leaves at their larval stage (Figure 1.4). Mulberry leaves is a traditional food for silkworm larvae due to presence of morin (Tribhuwan and Mathur, 1989). Human beings have benefited by the silkworm in various ways and scientists have been continuously trying to improve the techniques of silkworm rearing. The physiology of silkworm has been studied comprehensively due to its economically valuable silk production.

Mulberry belongs to the genus *Morus* under the family Moraceae. Three types of mulberry are found, white (*Morus alba* L.), red (*M. rubra*) and black mulberry (*M. nigra).* Among them, only white mulberry (*Morus alba* L.) is recognized as the food source for silkworm (Venkatesh and Chauhan, 2008).

The scientific classification of white mulberry is given below:

Kingdom: Plantae
(Unranked): Angiosperms
(Unranked): Eudicots
(Unranked): Rosids
Order: Rosales
Family: Moraceae
Genus: Morus
Species: *M. alba* L.

Mulberry leaves contain rutin, amino acid, nitrogen containing sugars, quercetin, different kinds of volatile oil, vitamins and many microelements, which have numerous pharmacological activities such as reducing blood glucose, hypertension, hyperlipidemia, viral contamination and also have bacteriostatic property (Zou and Chen, 2003). According to Ganesh Prabu *et al.* (2012) mulberry leaves contain several chemicals like 80% water, 27% proteins, 11% carbohydrates, vitamins and different minerals. Beside environment and technology adaptation, the nutritive values of mulberry leaves are a major factor for larval growth. The feeding quality of silkworm larvae was greatly influenced by foliar components such as moisture, carbohydrate, protein, mineral, fats, different amino acids and vitamins. The silkworm larvae are influenced by three stimulants in mulberry leaves viz. the attractant, biting factor and swallowing factor (Hamamura and Naito, 1961). Leaf consumption directly affects the silk producing capacity of the silkworm (Muthukrishnan *et al.,* 1978).

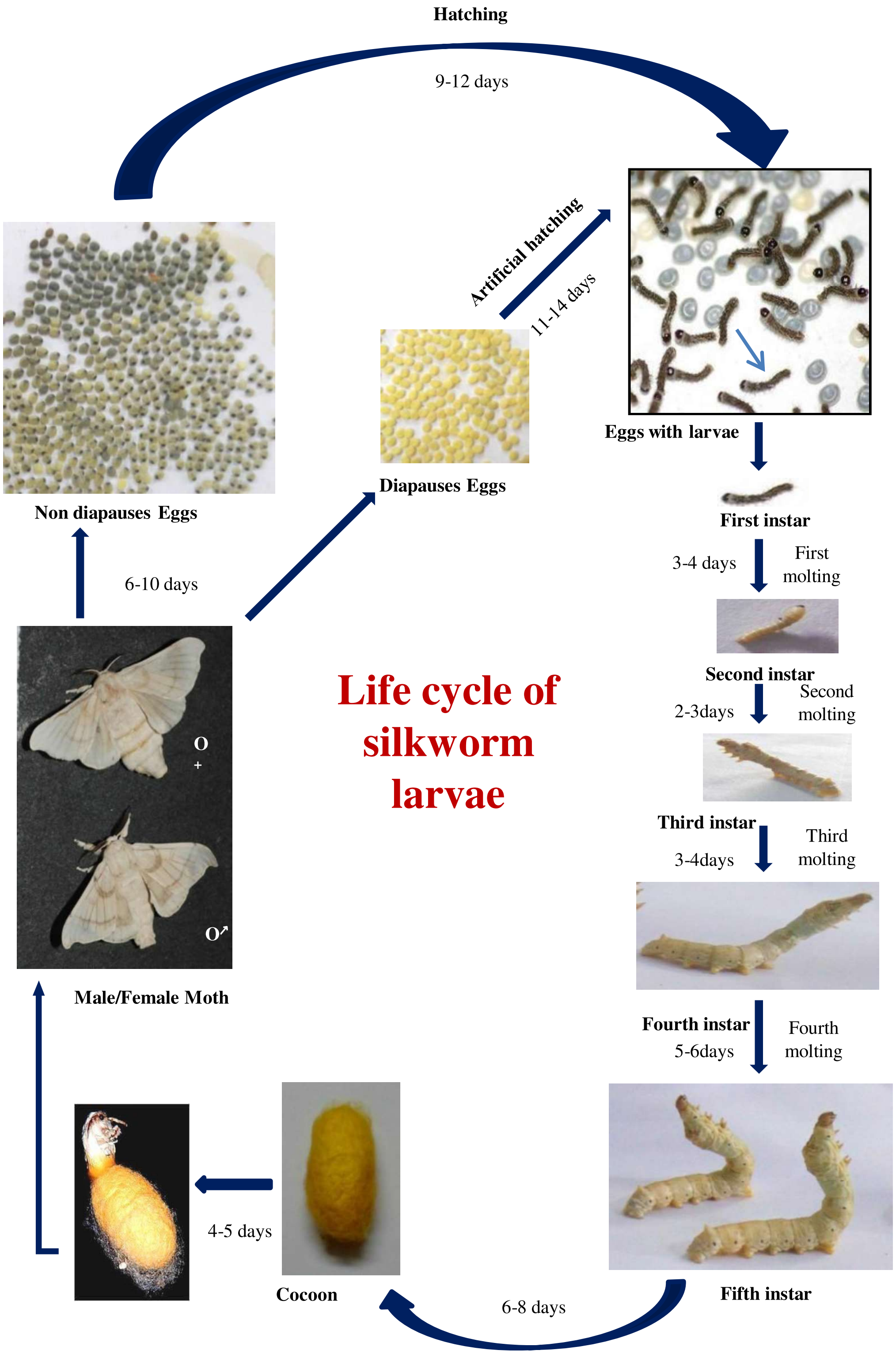

Figure 1.4: Different larval stage and overall life cycle of silkworm larvae

Sufficient documentation was not found for the explanation of choosing mulberry leaf by silkworm larvae. From literature survey, it seems that silkworm larval growth was totally dependent upon the quality of mulberry leaves. Besides leaf quality was differing with variation of mulberry genotypes. Therefore, selection of mulberry genotypes for silkworm rearing on the basis of leaf architecture and foliar nutrients seems to be a fundamental part of sericulture.

1.3 Feeding preference related with leaf architecture

The mulberry genotypes have been characterized on the basis of leaf surface attributes and serving as a powerful utility for mulberry improvement program, under tropical and sub-tropical climatic conditions. It was reported that features of leaf surface helps to determine the adaptability of mulberry genotypes with changing eco-climates (Southwood, 1986). Southwood (1986) also established a relationship between feeding quality of silkworm with mulberry leaf surface. Susheelamma and Jolly (1986) recognized a correlation between leaf stomatal size and stomatal frequency with adaptation of mulberry genotypes. Biasiolo *et al.* (2004) reported different micro morphological features of ten mulberry cultivars to describe and categorize them. In the recent past, stomata are a prime morphological feature of the leaves that have been studied from the taxonomical view point (Fagundez and Izco, 2011; Kaya *et al.* 2011). Venkatesh (2015) worked on micromorphology and karyotype of three mulberry genotypes and considered the stomatal features as an important attributes in mulberry genotype characterization. Moisture retention capacity was higher in those mulberry leaves possessing lower stomatal frequency with smaller in size (Basavaiah and Murthy, 2001).On the other hand, moisture content of mulberry leaves had a positive effects on silkworm larval growth and production (Paul *et al.,* 1992). Rahmathulla *et al.* (2006) correlates leaf moisture content with larval growth and silk production. According to Rahmathulla *et al.* (2006), higher leaf moisture leads to assimilate nutrients and more moisture build up in larval body, increasing productivity in sericulture.

Pattern of the trichome and idioblasts have been used for identification of mulberry genotypes in Japan (Fujita and Uchikawa, 1986). Presence of dense trichomes on the leaf surface causes mulberry leave’s unacceptability to the insects due to physical hindrance (Singh *et al.,* 1971; Levin, 1973). Baur *et al.* (1991) stated that trichome on leaf surface constitute a mechanical barrier that hinders herbivory insects feeding. Even leaf quality is superior on the aspect of its nutracutical properties, the leaf acceptability by silkworm larvae might be reduced with increasing trichomes density on the leaf surface (Kesavacharyulu *et al.,* 2004). Singhal *et al.* (2009) recognized Mandalaya, is promising mulberry genotypes for silkworm rearing due to high foliar yield and favorable micro morphological attributes of leaf such as lower trichome and idioblast density with shorter in length. Micromorphological SEM studies on leaf surface of different mulberry species namely K2, Kanva 2 (K2), *Morus laevigata* Wall., *M. multicaulis* Perrottet and *M. serrata* Rox. were observed by Kumar (2011). Also Kumar *et al.* (2012) worked on different attributes of leaf surface of four mulberry cultivars: S34, V1, TR10, and Mysore local. SEM study of leaf surface on *M. alba* also was studied by Yashvanth *et al.* (2015). Light microscope (LM) study was performed on leaf surface of *M. alba*, *M. laevigata*, *M. multicaulis, M. nigra*, and *M. rubra* L. (Philip *et al.,* 1989, Abbasi *et al.,* 2014). Klimko (2016) studied on micromorphology and anatomy of leaves of *Morus alba* cultivars and reported that abaxial leaf side in *M. alba* provides more diagnostic information about leaf surface than the adaxial one. Figure 1.5 represents overall survey of literature on feeding preference related with leaf architecture with respective references.

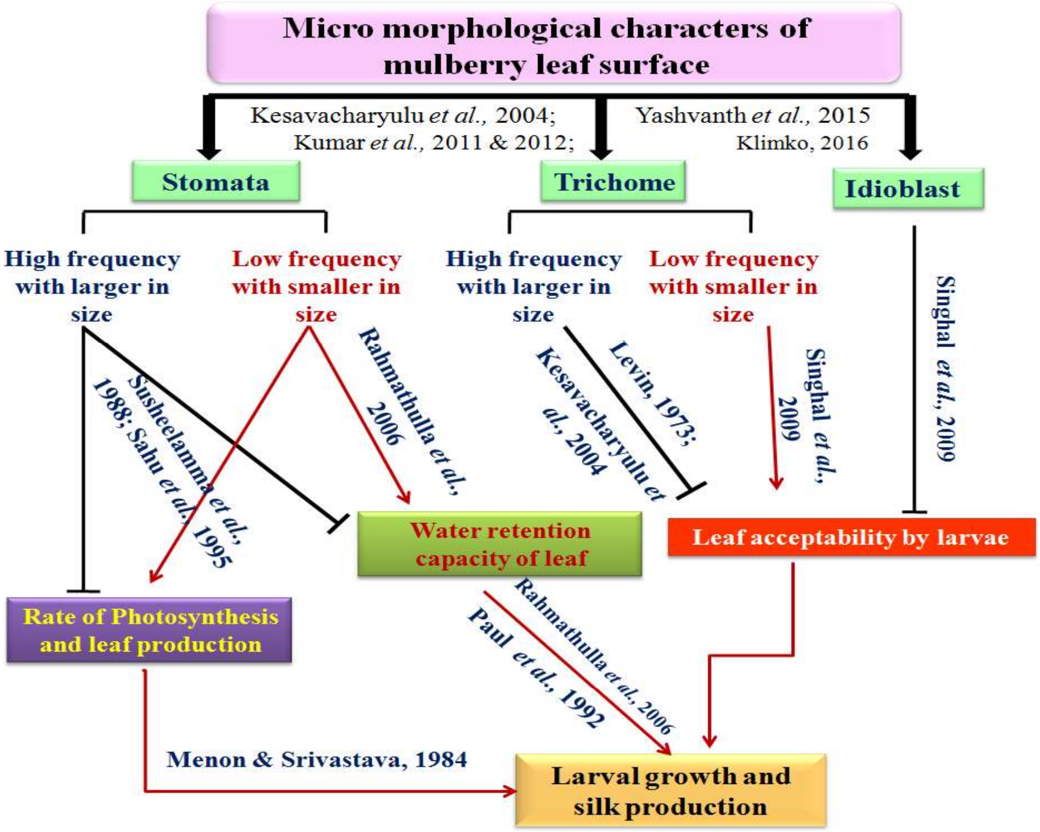

Figure 1.5: Schematic representation of literature survey on feeding preference related with leaf architecture

1.4 Feeding preference related with foliar nutrition

Optimal nutrient and favorable physical features are required for silkworm growth and silk production. Almost 70% silk proteins produced by silkworm are directly derived from the mulberry leaf protein (Narayanan *et al.,* 1967). Previous report clearly described that protein acts as essential ingredients in silkworm diet for larval growth and silk production. Arai and Ito (1967) established the presence of amino acid in the mulberry leaves protein can influence the larval growth. Elevation of dietary protein to an optional level (Horie *et al.,* 1971) and supplementation of low nutritive proteins with their limiting amino acids (Ito and Arai, 1965) have found to accelerate the growth of the silkworm. Rich sources of dietary proteins like soya protein are known to promote growth and improve the economic characters of the silkworm (Ito, 1980). Horie and Watanabe (1983) found soybean meal as a protein source in silkworm diet that can increase the weight of silkworm larvae and fresh silk glands. Krishnan *et al.* (1995) showed that the hydrolyzed soya-protein (P-soyatase) supplementation decreased the larval duration, increased the accumulation of haemolymph protein (SP-1: female specific protein and SP-2; an arlyphorin), larval weight and cocoon characters. Moustafa *et al.* (1997) worked on the nutritional effect of tested level of mulberry leaf powder and dietary soybean in the semi-artificial diet. El-Sayed (1999) and El-Hattab (2002) found the effects of dietary protein on larval growth and silk production.

For the growth and development of *Bombyx mori* L-ascorbic acid (vitamin C) has always been regarded as crucial factor. In fact, in mulberry leaves ascorbic acid is present in large amounts (Bresci, 1951; Lombardi, 1964), stimulatory effect of ascorbic acid was seen on silkworm voluntary feeding during the first instar stage (Ito, 1961). The larval development can be enhanced by vitamin C and B as coenzymes in amino-acid metabolism and antioxidant agents, which may increase amino-acid concentrations in larval tissues, leading to improvements in productivity. This hypothesis coincides with that of Babu *et al*. (1992), who observed that leaf consumption was greater in ascorbic-acid-fortified leaves as compared to control; practically vitamin C content of tissues and body fluids are highly dependent upon the amounts ingested with food. Vitamin B complex significantly improved larval growth and development and therefore vitamin B affects the economic features of the cocoon (Suprakash and Pal, 2002). This is supported by Sarker *et al*. (1995), who reported that supplementation of mulberry leaves offered to silkworm with ascorbic acid (1%) and vitamin B complex (0.5%) improved cocoon yield and silk filament quality. Bee-honey is also highly beneficial, improve rearing and the quality of the silk filament. Supplementation of mulberry leaves with vitamin B increased the resistance against poor environmental conditions and increased body weight in silkworm and vitamin B_{12} could increase the synthesis of nucleic acids and protein in the silk gland of silkworm (Das and Medda, 1988). Silk production is increased by Riboflavin which also reduces the choline and uric acid excretion and at end its derivatives are sprayed on mulberry leaf, thus helps silkworm to increase the fiber yield (Ito, 1978). Folic acid or folate is one of the vitamin B which is essential for the normal development of larvae's spine, brain and skull and also reported that it is necessary for nucleic acid biosynthesis in insects. It was also reported that dietary supplementation of folic acid to silkworm larvae did not significantly increase the glycogen content of the body but haemolymph trehalose content increases significantly (Nirwani and Kaliwal, 1996). Etebari (2002) showed that hyper vitaminosis does not only cause enhancement of larval and cocoon weight but it also has many negative effects too. Vitamin E enriched mulberry leaves did not have significant effect on food consumption rate in silkworm larvae (Mosallanejad *et al.,* 2002).

Salt may represent a limiting factor for the growth of insects, principally true for all types of diet composition (Wiggleswarth, 1972). The salt significantly improved the growth of the developmental stages, increased the cocoon characters, elicited early cocoon production and increased the reproductive potential of the silkworms. Nutritional supplementation of nickel chloride, potassium iodide and copper sulphate increased the economic parameters of the silkworm. It is reported that nickel chloride considerably increased the growth of silkworm larvae, pupae, adults and subsequently cocoon production but higher salt concentrations produced terminal effects on these parameters (Islam *et al.,* 2004). The cocoon weight was increased after feeding silkworm larvae with nickel and zinc fortified mulberry leaves (Chamudeswari and Radhakrishnaiah, 1994).

Insects require dietary sterols because there are unable to synthesize sterols *de novo* (Kircher, 1982; Nagata and Nagasawa, 2011). Sterols are crucial to insects for survival and development, since cholesterol is utilized not only as constituents of cell membranes, but also as precursors of molting hormones, ecdysteroids (Clarsk *et al.,* 1959; Grieneisen, 1994). Substantial sterol components of the midgut, epidermis, silk gland, and hemolymph of *Bombyx* larvae fed with mulberry leaves was reported much earlier on the basis of analytical records of preparative gas chromatography (Nayer and Fraenkel, 1962). β-sitosterol present in the mulberry leaves and conversion of β-sitosterol to cholesterol is crucial for *Bombyx* larvae (Nagata *et al.,* 2006). In addition to nutritional regulation of sterol requirements, β-sitosterol appears to contribute for activation of biting (Hamamura *et al.,* 1966) and dietary selection in this species (Nagata *et al.,* 2006). Therefore, sterol requirement of *B. mori* should provide important information on the relationship between nutritional regulation and feeding behaviour.

1.5 Effects of small protein or peptides on larval growth

On the other hand some scientist established the effects of few peptides isolated from silkworm larvae itself on the immune system. Moricin is a highly basic peptide, isolated from silkworm larvae, exhibited antimicrobial property by attacking the bacterial membrane (Hara and Yamakawa, 1995). A paralytic peptide (BmPP), a cytokine like factor which was purified from the silkworm hemolymph, have been reported to show multiple effects such as induction of morphological changes of plasmatocytes, inhibition of larval growth, promotion of cell growth, and local muscle contraction (Ha *et al.,* 1999; Sasagawa *et al.,* 2001; Miura *et al.,* 2002; Nakahara *et al.,* 2003).

In recent years a large number of biologically active peptides have been isolated from fungal, bacterial, plant and animal sources. Naturally occurring peptides are found to regulate several physiological processes in plants and animal system also. Among them, few are well characterized by scientist. Peptides in plant system play a definite role in amplifying signals, (Lindsey *et al.,* 2002), nitrogen fixation (Mylona *et al.,* 1995), cell proliferation (Matsubayashi and Sakagami, 1996) and generation of polarity (Souter and Lindsey, 2000). Not only plant system, peptides also regulate different metabolic activities and organ development of insects. Bombyxin, an insulin-related peptide, had an effects on the activity of glycogen phosphorylase, promotes cell proliferation, ovary development and involved in the pupal commitment of wing imaginal discs in different insects (Koyama *et al.,* 2008; Nijhout and Grunert, 2002; Mizoguchi and Okamoto, 2013). Few peptides had a role on oxidative stress management system. Amyloid β-peptide helps to reduce oxidative stress in human brain (Butterfield *et al.,* 2007). It may be possible that peptide from other source helps to reduce oxidative stress in silkworm larval midgut which is generated due to allelochemicals interaction with their host plant and also promotes larval growth. Proteomics study also was done on silkworm larvae after different treatment or elicitation.

Wang *et al.* (2004) used 2-DE and MS to examine the effects of lipo-polysaccharide injections on changes in polypeptides in the haemolymph, fat body and three portions of the midgut. Zhong *et al.* (2005) investigated the relationship between the 30K protein family and the embryonic development of a temperature-sensitive, sex-linked mutant strain of silkworm by 2-DE and MALDI-TOF/MS. Zhang *et al.* (2006) separated eight p25 isoforms of whole silk gland protein by 2-DE and identified them by peptide mass fingerprinting.

1.6 Various supplementary food used for silkworm rearing

Recently large number of investigations was done on the supplemented diet formulations based on mulberry leaves for silkworm rearing. The amino acid acts as a vital role in glucose, organic acid and tryptophan metabolism. Silk fibroin is derived from four amino acids such as alanine, glycine, serine and tyrosine (Kirimura, 1962). Ito (1983) demonstrated that silkworm obtained these four essential amino acids from their dietary protein and amino acid sources. Therefore, a small number of experiments were conducted on amino acid supplementation to improve silk cultivation (Etebari and Matindoost, 2005). Bhojne *et al.* (2013) worked on leaf supplementation with different amino acid on nutritional attributes of mulberry silkworm. Supplementation with certain amino acid viz. pectin and proline at low concentrations may be effective for silkworm larval growth (Bhojne *et al.,* 2013).

Multi-vitamin and mineral compounds could increase the food intake, growth and conversion efficiency of silkworm (Muniandy *et al.,* 1995). The group of vitamin B-complex and certain essential sugars, proteins, amino acids and minerals are responsible for the proper growth and development of the silkworm, *Bombyx mori* (Sengupta *et al.,* 1972 and Faruki, 1998). Fortification of mulberry leaves with vitamins as supplementary food compounds was carried out to increase silkworm larval growth and silk production (Saha and Khan, 1996; Nirwani *et al.,* 1998; Etebari *et al.,* 2004). Vitamins are required in minute amounts in diet that are essential for several metabolic activities in the cell to maintain proper growth and development. For normal growth of silkworm, vitamins are required in adequate amounts. Etebari *et al.* (2004) observed that production was decreased with increasing ascorbic acid concentration in larval diet. Rajabi Kanafi, (2007) concluded that one alternatives for improving larval feeding might be the enrichment of mulberry leaves with vitamins supplementation. Ahsan *et al.* (2013) demonstrated that low concentration of vitamin B and vitamin C had an impact on larval growth and silk production.

Dietary supplementation of copper sulphate, nickel chloride and potassium iodide increased the physiological attributes of the silkworm (Magadum, 1987). Ganesh Prabu *et al.* (2012) worked on food supplementation with silver nanoparticles on feeding efficacy of silkworm and reported that silver nano-particles can be used in sericulture to increase silk production.

There have been number of reports on supplementation of several kinds of plant growth regulators (PGRs) that affect growth, development and different physiological processes in different insects (Neumann, 1982; Chrominiske *et al.,* 1982; Bur, 1985). Silkworm larval growth and development was influenced by supplementation with plant hormones (Etebari *et al.,* 2004; Kochi and Kaliwal, 2005).

1.7 Elicitor mediated feeding response

The effect of different plant growth regulators (PGRs) on harvested mulberry leaves and their role on silkworm growth and subsequent cocoon production requires attention. But information is very inadequate in this respect. In recent times large number of PGRs is used as an enhancer in plant productions and also as signal molecules of different biological activities. Some PGR, especially, endogenous hormones of plant were introduced into herbivores insects through their diet. It has been reported that the PGRs may effect on neuroendocrine system of insects or directly on insects cell (Osborne *et al.,* 1968). Several works had been done on the effects of different PGRs on insects (Gurra, 1970; Alanso, 1971 and deMan *et al.,* 1991). There was evidence that PGRs had effects on molting hormone ecdysone and regulates *Drosophila hydei* growth (Alanso, 1971, Neumann, 1980). PGR acts as biochemical signals and helps to regulate insects' growth, their reproduction and DNA synthesis. Visscher (1980), more over stated that PGRs practically affect the productiveness, durability and egg vitality in insects.

Gibberellic acid (GA_3), a PGR derived in plant from acetyl Co A through mevalonic acid metabolic pathway. It plays a vital role in plant cell elongation, seed and bud dormancy breaking, seen germination, in flowering, reserves food mobilization in grass and sex expression (Salisbury and Ross, 1992). Plant growth rate, dry mass of plant and leaf area was enhanced by GA_3 spray on mustard plant. It was also documented that Gibberellic acid had a positive role on plant system as well as it affects economical parameters of silkworm rearing system with reduction of larval duration.

Animals are incapable to synthesize several essential hormones but they acquire it from several external sources, plants are one of them. Ecdysteroid hormones are that type of essential hormones for insects molting and oviposition. ABA is a ubiquitous plant hormone, chemically related with juvenile hormones. Juvenile hormones derived from external products, primarily ingested by herbivorous proto-insect. deMan *et al.* (1991) stated that biochemically similar PGRs like ABA and GA_3 can stimulate insect growth and their reproduction by altering synthesis of DNA and molting hormone together or separately.

According to Pramodkumari (1990), proteins of haemolymph and silk gland were affected by dietary supplementation with paraminobenzoic acid (PABA). Hugar and Kaliwal (1997) observed that body fat protein, fat glycogen and hemolymph protein were increased by tropical application with BAP and IAA. Silkworm fat protein, fat glycogen and hemolymph protein were increased after application with 2-4D and NOA (Goudar and Kaliwal, 2001). Bhattacharya *et al.* (2011) observed that indole-3-butyric acid (IBA) and indole-3-pyruvic acid (IPA) enhanced the economical attributes of silkworm rearing system viz. larval weight, silk gland weight, weight of the cocoon, shell and filament. Bhattacharya *et al.* (2011) also found that IBA, IPA and their synergistic effects on silkworm larval fat and haemolymph protein along with glycogen and trehalose content in their body fat and haemolymph respectively.

Cytokinin is a phytohormone, control the plant developmental programme and can also regulate plant responses against stress (Haberer and Kieber, 2002; Rivero *et al.,* 2007, 2010). It was also reported that cytokinin inhibited leaf senescence and promotes leaf nutrients (Zwack and Rashotte, 2013).

Kochi and Kaliwal (2005) found the effects of another phytohormone, salicylic acid on different commercial traits of silkworm larvae. There was significant increase in economic attributes of silkworm, after topical application with salicylic acid at different concentrations (Kochi and Kaliwal, 2005).

Polyamines were exhibited as important cell growth regulators and it controls different gene expression in various organisms (Tabor and Tabor, 1984; Pegg, 1988; Ha *et al.,* 1997; Cohen, 1998). To promote growth, spermine and spermidine are better than putrescine (Rouzina and Bloomfield, 1998). Polyamines play an essential role in silkworm development through cell proliferation, diapauses and metamorphosis (Renuka *et al.,* 2013). Yerra and Mamillapalli (2016) established the effects of polyamines on parental as well as hybrid strains of silkworm larvae. According to Yerra and Mamillapalli (2016), different economical parameters of silkworm rearing were influenced by spermine and spermidine treatment every day at 5th instars stage of larvae. Spermidine is a low molecular weight, essential cationic polyamine, required for growth of insect. It was reported that, rate of RNA and protein synthesis was increased with increasing spermidine concentration in *Drosophila*. Spermidine with micromole concentration had an effective role on tasar silkworm growth and increased the silk production (Renuka *et al.,* 2013).

Elicitor mediated silkworm rearing was documented by several researcher but the sufficient reports on elicitor mediated oxidative stress management is very little. Therefore, elicitor mediated oxidative stress management in silkworm yet to be under consideration.

1.8 Oxidative stress response

Reactive oxygen species (ROS) was generated during different biotic and abiotic stress in plant as well as insects body system. On the other hand, ROS additionally participate in ROS signaling pathway of different organism for adaptation to stress conditions (Krishnamurthy and Rathinasabapathi, 2013). Oxidative stress was generated due to several abiotic stress condition viz. salinity (Hernandez *et al.,* 1993), drought (Moran *et al.,* 1994), high temperature (Larkindale and Knight, 2002), heavy metal (Sytar *et al.,* 2013), and circumstances of biotic stress like allelopathic interaction of herbivory-host plant (Orozco-Cardenas and Ryan, 1999) and plant pathogen interaction (Grant *et al.,* 2000).

Oxidative stress leads to generation of ROS in intercellular place which ultimately leads the cell for protein oxidation. Protein oxidation can cause amino acid fragmentation. Peptides bond cleavage was found by ROS attack of glutamyl, aspartyl and prolyl side chains (Schuessler and Schilling, 1984; Uchida *et al.,* 1990). Garrison (1987) described that protein fragmentation occurs due to ROS attack at different side chain of ploypeptides chain.

Amino acid residues are susceptible to ROS. Sulfur containing amino acids viz. cysteine and methionine residues are partially sensitive to all kinds of ROS attack, but aromatic amino acid residues are the preferred intention for ROS (Berlett and Stadtman, 1997).

It was also noted that, protein content and nature of the protein in mulberry leaves affected at different stress condition. An antagonistic effect of salinity was found on proteins of mulberry leaves by breaking electrostatic bonds and increasing hydrophobic interactions

(Melander and Horvath, 1977). It was reported that high temperature had a role on the activity of photosynthetic enzymes and proteins in mulberry leaf (Chaitanya *et al.,* 2001). Total soluble protein content in mulberry leaves is decreased under high temperature and it also affects sugar metabolism through reduction in leaf starch content and sucrose-starch balance (Chaitanya *et al.,* 2001). High temperature is often associated with enhanced generation of reactive oxygen species (ROS) leading to cellular damage due to oxidative stress. Tewari *et al.* (2007) reported that oxidative stress was generated in young leaves of mulberry plants which were grown under nitrogen, phosphorus or potassium deficiency. Oxidative stress was generated through ROS generation and concerned redox couple in mulberry plants under Mn deficiency or Mn excess (Tewari *et al.,* 2013).

On the other hand, temperature is one of the important environmental factors that initiate physiological change in organisms. In mulberry silkworm *Bombyx mori*, high temperature is reported to exert adverse effect on testicular size and spermatogenesis, fertility and fecundity (Hussain *et al.,* 2011), growth rate (Kumar *et al.,* 2000) and efficiency of food utilization. Ramachandra *et al.* (2001) reported that silk worm larvae spun best cocoon at 22˚C. Some researcher showed that good quality cocoons were produced within 22–27˚C and cocoon quality was worsened at temperature level above these marks (Nabizadeh and Kumar, 2010).

Apart from the effects of high temperature on silkworm larvae, the presence of pesticide residue in mulberry may cause ROS generation and cellular damage. Oxidative stress also was generated into mid-gut of herbivorous insects due to allelopathic interaction with their respective host plant. Oxidative stress response in both plant and herbivore insects are explained in Figure. 1.6.

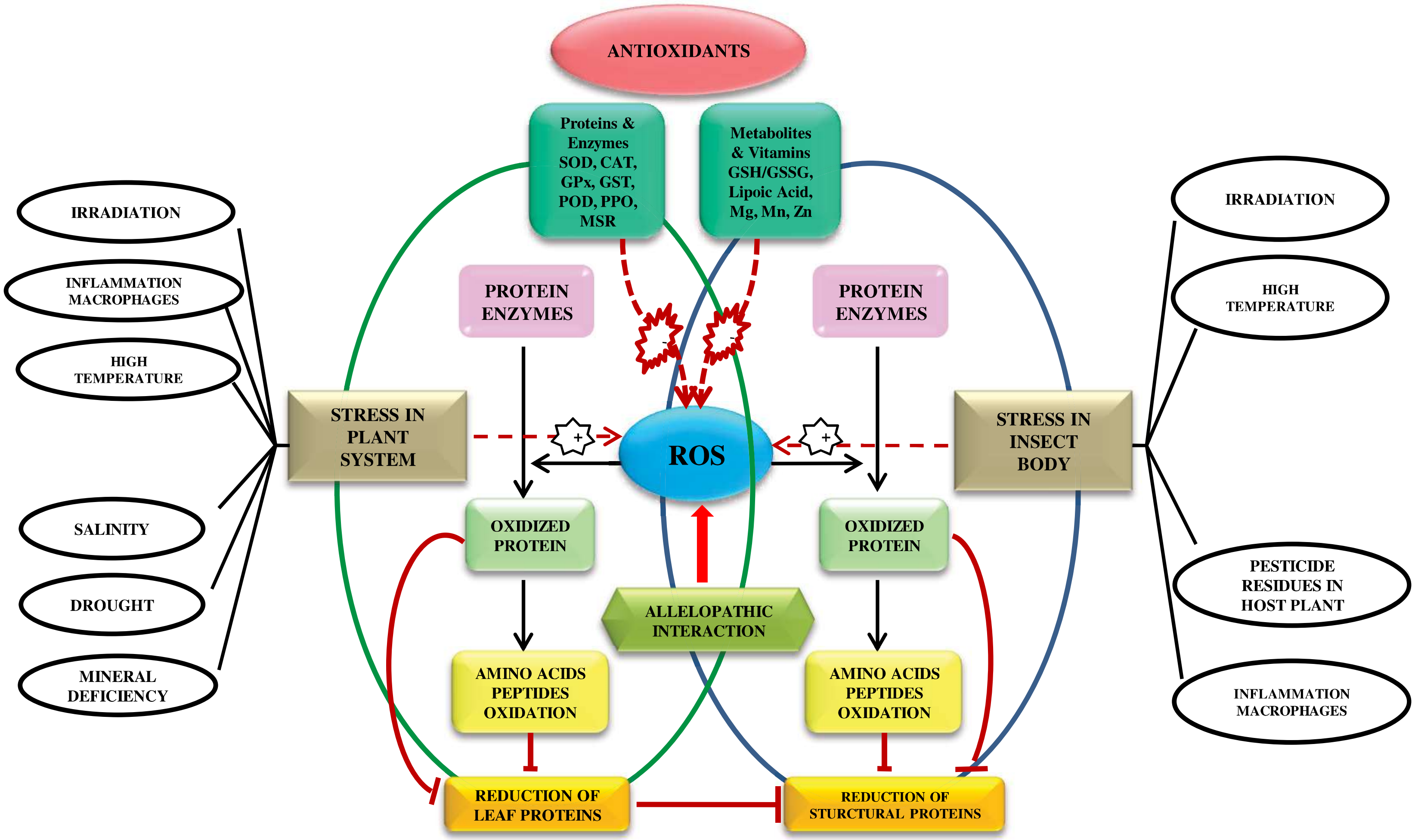

Figure 1.6: Oxidative stress response in herbivore insects and their host plant

1.9 Free radicals, Antioxidant molecules and free radical scavenging mechanism

Free radical can be defined as any independently subsisted molecule which have unpaired electron in its atomic orbital (Erbas and Sekerci, 2011). Free radicals are unstable due to presence of unpaired electron and are highly reactive. They have an ability to either donate or accept an electron from other molecules, hence behaving as an oxidant or reluctant molecules (Cheeseman and Slater, 1993). Once free radicals are generated into the cell, it will try to stabilize itself by gaining another electron from nearby molecule and the victim molecule becomes behave like a new free radical due to scarcity of an electron. Second newly generated free radical also will steal radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated from oxygen as by-products of different cellular redox process (Pham-Huy *et al.,* 2008). Reactive Oxygen Species (ROS) including several free radicals viz. hydroxyl radical ($OH^{\bullet}$), superoxide anion ($O_2^{\bullet -}$), peroxyl ($ROO^{\bullet}$) and also oxidants like hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), are constantly produced in the living organisms through oxidative metabolic pathways and also can lead to free radical reactions (Genestra, 2007; Pham-Huy *et al.,* 2008). Likewise, RNS such as nitric oxide radical ($NO^{\cdot}$), nitrogen dioxide ($NO_2^{\cdot}$), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$) are generated in the cell by oxidation of different organic substances (Droge, 2002; Valko *et al.,* 2007; and Pacher *et al.,* 2007). These free radicals are responsible for causing diseases like cancer, multiple sclerosis, diabetes mellitus, age related eye disease, inflammation, coronary heart disease, cardiovascular disease, arthritis, and neurodegenerative diseases such as Parkinson's disease etc (Harman, 1992; Rao *et al.,* 2006;). Proper imbalance of free radical generation and radical scavenging mechanism by antioxidant molecules leads the organism in an oxidative stress condition (Rock et al., 1996). Wide range of organic molecules including proteins, lipids and nucleic acids are affected by oxidative stress (Mc Cord, 2000). During oxidative stress, configuration of lipids and proteins molecules becomes changed by the process of oxidation. Antioxidant molecules can reduce oxidative stress and inhibit cellular damage by scavenging the effects of free radicals and thus, help in the prevention of various diseases (Halliwell, 1995).

An antioxidant is a molecule which can inhibit the oxidation of other molecules. Oxidation reaction is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce many free radicals. Chain reactions can be started by these radicals. When the chain reaction occurs in a cell, it can cause damage and sometimes death of the cell. Antioxidants terminate these kinds of chain reactions by removing free radical intermediates, and also inhibit other oxidation reactions. Antioxidants perform this by being oxidized themselves; therefore antioxidants are often reducing agents such as ascorbic acid, or polyphenols (Sies, 1997). According to Espinosa-Diez *et al.* (2015), antioxidant molecules are nucleophilic and reductant compounds which have ability to react with electrophilic oxidant giving them electron. Different enzymatic antioxidants including superoxide dismutase, catalase, glutathione, glutathione reductase, glutathione peroxidase, glutathione S-transferase, etc and non enzymatic antioxidants viz. glutathione, ubiquinol, vitamin C, vitamin E, β-carotene were found in organism with antioxidant properties (Levine *et al.* 1991). Some of them are synthesized in the organisms and rest one must be obtained from dietary sources. Ascorbic acid or vitamin C has antioxidant activity found in both plant and animal system. Glutathione is a peptide with cysteine residue found aerobic life forms (Meister and Anderson, 1983). The inhibitory response of proteins towards oxidation reactions makes them a vital component of an antioxidant based defence system.

In recent years, numerous naturally occurring bioactive peptides have been isolated from different living organisms which are found to be involved in the regulation of physiological processes in plants (Zhu *et al.,* 2006). Recently, the antioxidant potential of peptides derived from different plant samples has created a great attention among scientists. The peptides isolated from various plants like soybean (Moure *et al.,* 2006), maize (Moure *et al.,* 2006), chickpea (Li *et al.,* 2008), pea seed (Pownall *et al.,* 2010), buckwheat (Tang *et al.,* 2009), alfalfa leaves (Xie *et al.,* 2008) have been reported to possess potent antioxidant capacity.

1.10 Response of antioxidant enzyme

Free radicals cause oxidative stress leading to damage of bio-molecules and ultimately resulting cellular death if not properly eliminated (Hermes-Lima and Zenteno-Savin, 2002). Each organism has independent cellular antioxidant defense system which can protect cell against damage caused by oxidative stress.

Insects have own antioxidant defense mechanisms that consist of both enzymatic and non enzymatic components. The enzymatic antioxidants molecules are superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and Glutathion-S-transferase (GSTs). Relationship was established between GR and oxidative stress due to role of GR in the ROS releasing mechanism. GR reduced one GSSG to two GSH by utilizing FAD and NADPH. GPX1 is a ubiquitous enzyme of GPX family and can reduce cytosolic, mitochondrial and peroxisomal peroxides. GPX2 is an epithelial specific and highly abundant in intestine (Lubos *et al.,* 2011). GPX3 seems to be involved in the protection of cells from external peroxides (Lubos *et al.,* 2011). Unlike other GPX family members, GPX4 has broad substrate range in addition to H_2O_2 (Imai and Nakagawa, 2013). Glutathione-S-transferase is another important antioxidant enzymes family. The antioxidant activity of GSTs depends on the presence of GSH. SOD is another antioxidant enzyme which can catalyze the dismutation of the superoxide ($O_2^{\cdot -}$) radicals to hydrogen peroxide (H_2O_2) (McCord and Fridovich, 2014). CAT can stops to formation of $OH^{\cdot -}$ radicals by converting H_2O_2 to molecular oxygen and water (Dringen, 2000).

Herbivorous insect are challenged with ROS, produced within insects body due to pro-oxidant allelochemicals produced by host plants. Some herbivore insects have mid-gut based antioxidant defense against ROS. It has been reported that, insects can quickly elevated oxidative stress caused by plant pro-oxidant allelochemicals through antioxidant enzyme action (Krishnan and Kodrik, 2006). Mittapalli *et al.,* (2007) documented on enzymatic antioxidant defense response in Hessian fly and wheat plant interaction and reported that this type of antioxidant defense response may be applicable to other insect-host plant interactions. Jena *et al.* (2013) established a comparative documentation on total hydroperoxides and antioxidant defense system in the Tasar silkworm (*Antheraea mylitta*). Imbalance between ROS and antioxidants has an effect on different stages of life cycle and diapausing and non-diapausing of *Antheraea mylitta* (Jena *et al.,* 2013). Zhao and Shi (2009) observed significant variation in antioxidant enzymes including SOD, CAT, xanthin oxidase (XO) and H_2O_2 level in univoltine and polyvoltine strains of mulberry silkworm (*Bombyx mori*). Jena *et al.* (2014) also reported on the up-regulation of SOD, CAT and GST activity in midgut tissue of affected *A. mylitta* larvae. Sahoo *et al.* (2016) worked on pro-oxidant challenges and protection through antioxidant enzymes during larval development of Tasar silkworm. According to Sahoo *et al.* (2016), SOD and CAT are involved in protection of 1st instar larvae. Whereas combined enzymatic and non enzymatic antioxidant protection was reported during later developmental stages of larvae. Oxidative stress is generated during different developmental stages of caterpillar and depends on metabolic activity and feeding behavior of larvae. Accordingly, defense mechanism of enzymatic and non enzymatic antioxidants was modulated by the cellular redox signaling (Sahoo *et al.,* 2016). Significant increase of antioxidant enzymes such as SOD, CAT and GPx in haemolymph of mulberry silkworm were reported under various stressors (Micheal and Subramanyam, 2014). According to Micheal and Subramanyam (2014), the antioxidant activity of SOD and CAT depends on larval stages. However, no relationship was found between GPx activity with silkworm larval stage or larval age. Enzymatic antioxidant marker namely SOD, GST, GPx, and GR in lower frequency were involved in antioxidant defense mechanism in response to exposure of dichlorvos in mulberry silkworm, *Bombyx mori* (Muthusamy and Rajakumar, 2016).

Oxidative stress was generated in plant system due to different biotic and abiotic stresses. Chloroplast, mitochondria and peroxisomes are major intercellular organ where ROS are generated (Rich and Bonner, 1978). Plants also have antioxidant defense system possess low molecular weight antioxidants viz. ascorbate, glutathione, carotenoids as well as antioxidant enzymes. Peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT) acts as the major antioxidant enzymes against ROS produced in different parts of plants cells (Apel and Hirt, 2004). Another important antioxidant enzyme is polyphenol oxidase (PPO) which can oxidize several phenols to chinone. PPO had been reported in plenty of plants. Demir and Kocacaliskan, (2001) reported on PPO present in plant in relation to enzymatic browning. Presence and activity of PPO in relation to biosynthesis of alkaloids under several biotic and abiotic stresses also was reported (Bilkova *et al.,* 2005). It has been reported that activity of different antioxidant enzyme was changed under stress condition (Hernandez *et al.,* 2000; Koskeroglu and Tuna, 2008; Venkatesan and Sridevi, 2009). Sudhakar *et al.* (2001) observed the changes of antioxidant enzyme efficacy in different mulberry genotypes under NaCl salinity. High accumulation of ROS in mulberry plants under salinity and high temperature stress was reported and activity of POD, SOD and APX was increased in response to stress condition (Yu *et al.,* 2013). Chaitanya *et al.* (2002) observed variation among antioxidant enzyme activities in mulberry cultivars under heat stress. Mulberry plants can increased their resistance against oxidative stress by enhancing the activities of POD, SOD and ascorbate-glutathione cycle (Harinasut *et al.,* 2003).

A lot of information is available on the response of different antioxidant enzymes in silkworm larvae as well as their host plant. However, little is known about antioxidant enzymes of larvae influenced by plant antioxidant defense system.

1.11 Antioxidant activities of mulberry leaves

Morus alba was found to possess potential antioxidant properties which are reported by various researchers (Chu *et al*., 2006, Katsube *et al*., 2006, Bae and Suh, 2007, Ercisli and Orhan, 2008). Plants have phenolic compounds with antioxidant activity (Linghong, 2012). Zou *et al.* (2012) isolated four major phenolics including benzoic acid, chlorogenic acid, astragalin and rutin from mulberry leaves and quantified the phenolics using HPLC-UV method. According to Zou *et al.* (2012), phenolics content and antioxidant activities of mulberry leaves depend on mulberry cultivars and harvesting season. Khyade (2016) analyzed the elevated antioxidant activity of phenolic compounds from methanolic extract of mulberry leaves. High correlation and regression was found between phenolic contents and antioxidant potentials of methanolic extracts from different parts of mulberry (Khan *et al.,* 2013). Enkhmaa *et al.* (2005) detected flavonoids glycoside in mulberry leaf, can acts as a potent antioxidant. Three major flavonol glycosides such as quercetin 3-(6-malonylglucoside), rutin and isoquercitrin were identified by LC-MS and NMR from mulberry leaves, had low density lipoprotein (LDL) antioxidant compounds (Katsube *et al.,* 2006). Aqueous extract of mulberry leaves contains antioxidative substances that might prevent atherosclerosis (Harauma *et al.,* 2007). Naowaboot *et al.* (2009) reported antihyperglycemic, antioxidant and antiglycation activities of mulberry leaves in streptozotocin induced diabetic rats. Mulberry leaves extracted by different solvent also was exhibited free radical scavenging activities. Mulberry leaf extracted by methanol or butanol had better radical scavenging activities in the comparison with aqueous extracts (Naowaratwattana *et al.,* 2010). Aramwit *et al.* (2013) exhibited that mulberry leaf powder had strong antioxidant activity. Mulberry leaf extract exhibited antioxidant activity was measured by DPPH and ABTS radical scavenging activities (Flaczyk *et al.,* 2013). Mulberry leaf extract had antioxidant activities as well as hypoglycaemic and androgenic properties in diabetic rats (Hajizadeh *et al.,* 2014). Samuel *et al.* (2016) reported that mulberry leaf extracts seem to possess potent antioxidant activity and can be used as food supplement.

However, several secondary metabolites namely polyphenol and flavonols, present in mulberry leaves had antioxidant activities but many others molecules with antioxidant properties are still unknown or are yet to be investigated.

1.12 Elicitor mediated oxidative stress management

Plants are challenged by variety of stresses and activate separate defense pathway accordingly with respective stressors. Hormonal signal seem to be initiated by different biotic stressor. Jasmonic acid, ethylene, salicylic acid dependent signaling is activated by such biotic stressors. Several natural and synthetic compounds called elicitors play a vital role to understand different plant signaling pathways in plant system (Gomez-Vasquez *et al.,* 2004). Originally the term "elicitor" is defined by the molecules responsible for introducing different phytoalexins, but the term is now commonly used for compounds that help to stimulate any kind of plant defence (Ebel and Cosio, 1994; Hahn, 1996; Nurnberger, 1999). According to Duenas *et al.* (2015), elicitors can alter physiological processes in plant system by enhancing various bioactive components. Elicitors are classified on the basis of their origin, molecular structure and mode of action. The origin of elicitors may be biotic or abiotic, physical or chemical and the mode of action may be complex or defined (Thakur and Sohal, 2013). Elicitors can be categorised into two groups: general elicitors which can trigger their action in both host and non host plants, another is race specific elicitors express their specific function in specific host plants only (Thakur and Sohal, 2013). Elicitors including different macro and micro-molecules namely carbohydrate, lipids, glycoproteins, glycopeptides and polymers had been characterized in plant kingdom. Elicitor acts as a signal molecule at low concentration and responsible for elicitor mediated signal transduction mechanism in plant to respond under different stresses. Elicitation is a process caused by the elicitor application in organism and it induced or enhanced synthesis of secondary metabolites to ensure better response of organism under different environmental condition (Patel and Krishnamurthy, 2013).

Several plant hormones have been studied as an effective elicitor due to their key roles in plant defense system. Gibberellic acid is an important phytohormone and used as an effective elicitors, responsible for secondary metabolites production in plant (Liang *et al.,* 2013). Salicylic acid (SA) also can elicit the production of secondary metabolites in plant and it was reported that SA affects terpenoids metabolism in plant (Naik and Khayri, 2016). Activity of antioxidant enzymes such as SOD, POD, and APX were increased by SA application in maize plant under saline condition (Fahad and Bano, 2012). Jasmonic acid (JA) is important elicitors for several plant secondary metabolic pathways (Pauwels *et al.,* 2009) and acts as a vital signalling molecule in response of different stress (Wasternack and Parthier, 1997). Mejia-Teniente *et al.* (2013)

reported that exogenous application of SA, H_2O_2 and chitosan (QN) elicitors in *Capsicum annuum* significantly increased gene expression and antioxidant enzyme activities related with plant defense. Abscisic acid (ABA) is a natural growth regulator and acts as a significant elicitor to stimulate the antioxidant defense system in plant. ABA enhanced antioxidant capacity and protects plants from various abiotic stresses (Ding *et al.,* 2010; Wang *et al.,* 2011). Application of ABA induced SOD enzyme activity to the response of drought stress (Wang *et al.,* 2011). According to Jiang and Zhang (2001), ABA stimulates antioxidant defense system by enhancing generation of antioxidant molecules which can resist the oxidative stress. Activity of antioxidant enzyme namely APX and SOD was increased in *Syzygium cumini* plant by ABA and H_2O_2 elicitation (Choudhary *et al.,* 2012). Exogenous ABA application enhanced the SOD and POD activity and the expression of genes related to the AsA-GSH cycle in pepper plant also was increased under chilling stress (Guo *et al.,* 2012). It has been reported that ABA significantly increased the POD activity in tomato plant (Basak *et al.,* 2012). Exogenous application of ABA increased antioxidant enzymes POD and ACP activity in *Pisum sativum* under drought stress (Latif, 2014). Nedeva *et al.* (2008) observed the effect of different plant growth regulators namely ABA, fluridone, methyl jasmonate, SA and cytokinin on antioxidant isoenzymes in response to stress.

The activities of antioxidant enzyme specially POD and SOD activity was enhanced after Ascorbic acid (AA) elicitation in sugarcane plant (Ejaz *et al.,* 2012). Ascorbic acid is an effective growth regulator against different stresses (Conklin, 2001). An increase in antioxidant enzyme activity by exogenous application with AA was reported in wheat plant (Athar *et al.,* 2008, 2009).

Polyamines are also used as an elicitor and it is an organic polycationic unsaturated hydrocarbon. Response of abiotic and biotic stress is modulated by polyamines (Kumar *et al.,* 1997). Polyamines are involved in various cellular processes such as protein synthesis, transcription, RNA modification and modulation of enzyme activity (Tabor and Tabor, 1999). However, very few evidence was recorded on effective role of polyamines in up and down regulation of physiological activities under stress condition (Alcazar *et al.,* 2010). Elicitation through polyamine in *Salvinia natans* reduced the generation and accumulation of H_2O_2 and also modulated the antioxidant enzyme activities (Mandal *et al.,* 2013).

Being a rich source of nutrient, silkworm larvae selects mulberry leaves as a prime source of food. Through ongoing survey of literature, it was found that several studies were concentrated mainly on different supplemented food of silkworm along with the effects of different isolated proteins on silkworm growth but till now no report has been established on the effects of isolated plant or animal peptides in silkworm rearing. Silkworm larvae have been reported to face oxidative stress due to allelopathic interaction with their host plant. Silkworm larvae possess various kinds of antioxidant enzymes to reduce stress. Therefore, mulberry leaves have also significant antioxidant defense mechanism to reduce oxidative stress. But the interaction between antioxidant mechanism of plant and insect are yet to be established. Through external application of additional source of antioxidants, the effects of foliar antioxidants on silkworm larval growth is yet to be investigated under normal or oxidative stressed condition, particularly at tropical belt where the temperature regime goes beyond 35° C during summer season. Several techniques have been introduced by researchers regarding the enhancement in the nutritional as well as antioxidant components of various plants among which elicitation has been considered as an effective technique. Various kinds of elicitors have been reported to enhance biological activity in plants. Therefore, the present study have been carried out to explore the role of several hormone as well as low molecular plant peptides as a source of elicitor on silkworm oxidative stress management and which ultimately will enhance the productivity in sericulture.