SUMMARY

The present study deal with the investigation of biochemical attributes of mulberry leaves and silkworm rearing system through elicitation by peptides and other growth regulators. The thesis was configured with nine chapters excluding 'General Introduction' and 'Summary'. An extensive review of literatures was done covering various aspects of the relevant topic prior to the commencement of the research work. The review was started with global, national and state scenario of sericulture. From the literature cited, it was found that silkworm feeding behaviour was related with the micro-morphological and phytochemical attributes of mulberry leaves. The beneficial roles of elicitor including small protein or low molecular weight peptides, plant hormones, polyamines, non-enzymatic antioxidant, inorganic salt and other plant growth regulators at their optimized concentration and their cellular signal governing essential physiology were initially highlighted in the review. According to literature, various supplementary foods were used beneficial for silkworm rearing since ancient times. Further, the review highlights on concept of free radicals, their mode of actions and their effects on the biological system. Antioxidant is the compound responsible for combating against damage caused by free radicals and the oxidative stress. Additionally, the review section comprised of the various mode of elicitors and their relevance in enhancement of bioactive metabolites in mulberry plants for functionally assisting oxidative stress management in silkworm larval system.

The rest of the chapters were written on the basis of work performed for executing the objectives of the present work. Chapter II mainly deals with the screening of most tolerant and suitable mulberry cultivars for silkworm rearing. Seven mulberry cultivars were primarily selected namely S1, S1635, V1, K2, Kosen, Bombay Local and Dudhiya for the study. On the basis of micro-morphological features S1 and S1635 were most promising mulberry cultivars with high foliar yield and high acceptability by silkworm larvae. Acceptability of different mulberry cultivars by larvae was measured by different economical attributes of silkworm rearing. S1 and S1635 both had shorter trichome length with low trichome and idioblast density ultimately helped the cultivars being suitable for larval feeding. Low stomatal frequency with short stomatal size was also favourable leaf surface attribute of these promising mulberry cultivars. Suitable stomatal features influenced foliar yield and nutrition, which influence the larval growth and silk production. Imbalance in foliar nutrients affected larval metabolic activities. Highest larval growth rate was recorded under nourishment with S1 leaves. Effective rearing rate is a commercial character, reflects on success rate of rearing and production rate. Larvae reared with S1 and S1635 mulberry leaves, exhibited highest EER (%) and minimal (40%) ERR was recorded by Dudhiya nourishment. Shell ratio (SR %) helps to determine the amount

of raw silk obtained from fresh cocoons. Highest SR % was obtained with S1 nourishment. The study revealed that K2 and V1 also can be selected for silkworm rearing in sub-tropical region of India.

The genotypic selection was mentioned in chapter III, on the basis of leaf nutritional values and feeding preference by larvae associated with seasonal variation. Biochemical component of leaves depend on various factors, namely cultivars or genotypes, soil nutrients, water, cultural practices and seasonal variation. Plants suffer various stresses at different seasons. During winter season, mulberry plants experience serious osmotic stress due to a significant drop of water potential in soil. Due to excess rain, the flood situation and hypoxic stress again might be created during rainy season. During this time soil contains low microelements. Mulberry plants also suffer oxidative stress due to deficiency of microelements like Mn, N, P, and K in soil. Oxidative stress parameters of mulberry leaves were evaluated by considering the seasonal fluctuations of free radical accumulation in the plant body. The mulberry genotypes were categorized into two classes: acclimated and non-acclimated. Acclimated genotypes are those who can build up high free radical scavengers and reduces free radicals accumulation during the stress periods. If the genotypic responses in this situation were considered, S1, V1 and S1635 variety could successfully manage the minimum accumulation of free radicals like peroxide, superoxide and MDA. Besides this, ascorbic acid and glutathione were sufficiently accumulated in V1 and S1 cultivars, but this accumulation was significantly lesser in Dudhiya genotypes. The findings indicated that the glutathione-ascorbate pool gave sufficient feedback for the regeneration of other antioxidant molecules among stress tolerant cultivars like V1, S1 and S1635 during the crisis. Silkworm larvae, particularly rejects the Dudhiya germplasm due to the accumulation of excess ROS and peroxidized product generated from membrane lipid during the stress period. In contrast, nutritional and antioxidant rich genotypes were preferred by larvae. Homeostatic action between ROS production and scavenging activities might facilitate proper ROS signalling, which can directly or indirectly help in maintaining optimum plant protein and carbohydrate production. Lastly, S1, V1 and S1635 may be recommended for commercial cultivation for better silkworm rearing by nourishing S1, S1635 and V1 leaves.

Next study deals with the mulberry leaves elicitation technique with low molecular weight (0.5-3 and 3-10 kDa) peptides. Elicitation is a good approach to induce changes in physiological and metabolic activity and stimulate stress induced or defence responses in plants. Peptides were isolated from leaves of relatively tolerant cultivars like S1, S1635 and V1 and one vulnerable germplasm Dudhiya as control. 5^{th} instar larval growth rate pattern was found to be improved under the influence

of different peptide(s) treatment. Consumption rate of the larvae under peptide(s) treatment was increased significantly over control. Low molecular weight peptides (0.5-3 kDa) exhibited better performance in comparison with high molecular range (3-10 kDa) peptides in all treatments. Significant difference in the shell ratio (%) occurred with S1 peptide(s) application in comparison with other peptide elicitation. Application of peptides helped to maintain larval growth and all economical attributes under various environmental circumstances. Peptide segments of 0.5 -3 kDa and 3-10 kDa proteins can stimulate a complex mechanism in mulberry leaves which helps to maintain different metabolic pathway in proper way throughout all environmental situation. It may be possible that, peptides can help to develop a defence network in mulberry leaves under different environmental circumstances. Peptide elicitors can bind with plant cell membrane, exceed into larval body through mulberry leaf and trigger the larval metabolic activity, increase the juvenile hormones biosynthesis and ultimately enhance the silk production.

Peptides isolated from different mulberry leaves had potentiality to scavenge free radicals. Antioxidant response of peptides was molecular weight dependent manner. Low molecular weight (0.5-3 kDa) peptides had high antioxidant capacity in comparison with high molecular weight (3-10 kDa) ranges. Antioxidant activity of peptides also was source dependent. Peptides isolated from S1 mulberry leaves had better ROS scavenging potentiality than peptides isolated from S1635, V1 and Dudhiya leaves. Antioxidant rich peptides could function as a beneficial supplementary nutraceuticals with mulberry leaves to oxidative stress management in silkworm larvae. Free radicals were generated into midgut of herbivore silkworm larvae due to allelopathic interaction with host plant. Degradation of phenolics of host plants into midgut of insects generates ROS. Peptides increased larval antioxidant enzymes to reduce oxidative damage in different cellular parts; while Dudhiya leaves were refused by silkworm larvae, the peptides isolated from Dudhiya could influence antioxidant enzymes in silkworm larvae. But the quantum of improvement of antioxidant enzymes was comparatively lower than other source of peptides. This study opens a scope to maintain oxidative stress in other herbivore insects or other silkworm such as Tasar, Muga and Eri by peptides elicitation or by peptides supplemented with their respective host plant.

After successful silkworm rearing through bioactive peptides elicitation in mulberry leaves; the well known post cocoon attributes of silkworm rearing system was targeted for further enhancement. Therefore, the large scale field trail was organized to determine the quality of silk fibre on the basis of economical aspect. The considerable enhancement in shell ratio, average filament length, non-

breakable filament length, and silk protein: fibroin and sericin induced the idea for application of peptide in sericulture to enhance productivity for commercial purpose.

Next the present investigation deals with the purification and characterization of low molecular weight mulberry peptides. TLC analysis was performed with low molecular weight (LMW) especially 0.5-3 kDa oligopeptides because strong bioactivity was acquired with LMW peptide fractions. Isolated mulberry peptides are diverse peptides differing in their amino acid composition that display antioxidant potentiality. The amino acid composition differs with the variation of mulberry genotypes. Profile study of bioactive peptides through thin layer chromatography revealed that several small peptides were degenerated during maturation of mulberry leaves. Low molecular weight oligopeptides were extensively purified by C18 RP-HPLC. The formation of multiple peaks for compositionally pure peptide sample might be attributed to presence of multiple smaller peptide chains which was denaturised and separated during purification process using RP-HPLC. Complex oligopeptides was separated and amino acid sequencing was carried out for finding peptide structure. The presence of aromatic, hydrophobic and heavy amino acids like Tyr, Phe, Ala were responsible for antioxidant potentiality of mulberry peptides.

Chapter VIII and IX was configured with elicitor mediated feeding response of silkworm larvae with various plant growth regulators (PGRs) and PGR mediated oxidative stress management. Initially, plant hormones (ABA, GA, IAA and Kinetin), polyamine (Spermine, spermidine, putrescine); non-enzymatic antioxidant (ascorbic acid and glutathione); compatible osmolyte (Proline), folic acid (vitamin B9) and inorganic salt (nickel chloride and sodium chloride) were used for mulberry leaves elicitation. Finally abscisic acid, gibberellic acid, spermidine and ascorbic acid were selected for large scale field performance as better response was shown by them in laboratory scale considering economical aspects. PGRs enhanced shell ratio, length of the bave, average single filament length (mt) in comparison with control. The defective percentage directly affects the raw silk quality. PGRs application reduced defects on silk filaments. Reelability is considered as the fitness of cocoons in commercial aspect. Renditta is another essential commercial reeling characteristic of silk. Low renditta reflects the high quality of cocoons. PGRs lowered the renditta of cocoon in comparison with control. Waste percentage is the percentage of raw silk amount which is unable to wind in silk production. Waste is the floss of upper most layer of thread comes out during reeling and expressed as waste percentage. Waste percentage was significantly reduced by PGRs application over control. Exogenous application of PGRs increases antioxidant accumulation in the foliar system which

ultimately helps in balancing ROS/antioxidant ratio. Proper ration of ROS/antioxidant molecule stimulates metabolic activities of plant system. Foliar nutrition was maintained during different season after PGRs elicitation. However, the synergistic action of PGRs to maintain redox status in larval body and their precise involvement in silk production is still unclear. Therefore, these PGRs at their respective concentrations might be recommended for foliar application of mulberry leaves to enhance silk production.

Lastly, it might be concluded that mulberry leaf peptides are a very low molecular weight molecules had strong regulatory effects on silkworm rearing and also improved physical and biochemical properties of the silk. The molecular association with other physiological and metabolically sequence of the silkworm will be a good futuristic study. A more detailed fluorescent analysis for mode of binding of low molecular weight peptides and transition of peptide from leaf to larvae needs to be undertaken for more elaborate understanding and clarification of the present observation. In near future, further studies are needed for the sole characterization of the molecule so that it would stand as economical but promotive molecule for the future silk industry.