8. SUMMARY

Silk fibers obtained from cocoons that are spinned by *Bombyx mori* are considered as best quality silk. India ranks 2nd in silk production, with an average annual production of ~28,000 metric tons after China. The silk industry in India is mostly agro-based, depending upon traditional practitioners for fulfilling the national and global demand of raw silk. Superior quality silk production depends upon quality of mulberry leaves larvae fed upon. The three most significant leaf parameters upon which entire raring practice and thereby production of good quality cocoons depends are moisture, protein and carbohydrate content. Moisture helps to maintain proper leaf texture (suitable for larval chewing), while carbohydrate provides energy for larval growth and molting, as well as during spinning of cocoons. The mulberry leaf protein that gets assimilated inside the body of the larvae helps in the development of silk gland which ultimately leads to the development of good quality cocoons.

The practice of silk industry consist of two segments *viz*. cultivation of mulberry (outdoor segment) and raring of larvae (indoor segment). Cultivation of mulberry requires open land, restricting sericulture practice to those farmers bearing large to small scale agricultural lands. Landless farmers either work on others' farm in daily wedge system or if they are willing to run their own practice they purchase leaves from other's garden, increasing the cost of production. With increase in demand of labours in urban cities, landless farmers often migrate to large cities leaving their traditional practice.

Another problem that was faced by silk producing farmers across the country was feeding of larvae during monsoon season. During monsoon, feeding wet leaves led to the development of larval disease, increase the rate of mortality and thereby production of raw silk decreases.

Leaf preservation by retaining the natural physical and chemical nature of leaves using suitable preservative may help to solve the above stated problems; as on one side landless farmers may carry out raring practice by collecting leaves once in a while and on the other side during monsoon season harvesting and preserving leaves on a non-rainy day may sort the problem of increasing larval mortality. The challenges which are faced during leaf preservation, putting a limit over prolongation

of shelf life are wilting, discoloration, senescence, enhanced rate of respiration, tissue browning, decay and microbial proliferation.

Out of the nine chemicals initially selected as preservative, silver nitrate showed potentiality to enhance the shelf life of cultivated mulberry leaves by seven days. During the course of experiment it was observed that silver nitrate solution gradually gets transformed to nanosilver with increase in days of preservation, which ultimately prolongs the shelf life. Thus current study was designed to observe the preservative effect of green synthesized (phytosynthesized) nanosilver by comparing its preservative potentiality with silver nitrate, serving as positive control and distilled water serving as negative control. Nanosilver preserved leaves showed significant retention of primary metabolites *viz.* total chlorophyll, protein, carbohydrate and many others till the last day of preservation. Retention of primary metabolites during the course of preservation was also displayed by leaves preserved in silver nitrate solution but the degree of retention was comparatively lesser than that displayed by nanosilver preserved leaves. Leaves preserved in distilled water showed high accumulation of free radicals causing oxidative damage along with significantly lesser content of primary and essential secondary metabolites and defensive proteins. Upliftment of stress content was also noted in nanosilver and silver nitrate sets but extent of enhancement was significantly lesser due to the presence of enhanced enzymatic (CAT, SOD, APX, GSR, GST, GPOX) and non enzymatic (glutathione, ascorbate and carotenoids) defensive activities. Nanosilver solution showed almost equivalent potentiality to extend the shelf life of all studied cultivars of mulberry *viz.* S1, S1635, BC259, TR10 and Guangdong, with S1 displaying best post-harvest retention of essential metabolites after preservation.

Macro-molecule (protein, lignin and suberin) mediated xylem blockage inhibiting normal conducting pathway was found to be one of the principal delimitations inhibiting post-harvest shelf life extensions. Another obstacle inhibiting post-harvest shelf life was proliferation of microorganisms within the preservative solution, which ultimately causes obstacle inside xylem vessels, accelerating the process of senescence. Nanosilver solution showed strong antimicrobial activity and thus no CFU count was noted within the nanosilver solution used for prolonging shelf life of mulberry during the entire course of preservation. Histochemical based microscopic observation revealed insignificant number of blocked vessels in the transverse section

of petiole preserved in nanosilver solution. PCA and AHC based statistical analysis showed close proximity of nanosilver preserved leaves with freshly collected leaves, signifying preservative potentiality of nanosilver solution. Phytosynthesized nanosilver also displayed strong antioxidant activity, probably playing an indirect role in mitigating generated biotic stress. It was observed that nanosilver solution at 6 ppm concentration was the least effective concentration suitable for prolonging shelf life of mulberry leaves by 7 days.

Silkworm larvae on feeding nanosilver preserved leaves showed almost equivalent feeding profile to that of larvae supplemented with fresh leaves. Depletion in feeding profile was noted for larvae supplemented with silver nitrate and distilled water preserved leaves in comparison to larvae supplemented with fresh leaves, but the degree of depletion was found to be insignificant for silver nitrate set, while that for distilled water set it was found to be highly significant. Larvae fed with leaves preserved in nanosilver solution showed comparatively less mortality rate and high cocoon and cell weight than other preservative sets, indicating the non-toxic nature of biosynthesized nanosilver solution.

Electrophoresis based protein banding pattern displayed uniform retention of protein bands till the last day of preservation in nanosilver preserved leaves, while depletion in band intensity was noted in silver nitrate and distilled water preserved leaves from 5^{th} and 3^{rd} day onwards respectively. Uniform protein banding pattern was also noted during electrophoretic separation of silk gland, haemolymph, stomach and fat body proteins in larvae of nanosilver set, indicating normal body physiology of larvae supplemented with nanosilver preserved leaves. Preservative potentiality of nanosilver and silver nitrate solution was also revealed through isozyme profiling which showed enhanced activity of stress mitigating isozymes like CAT and SOD in both preserved leaves and silk gland of larvae fed with preserved leaves.

Transcriptome based Illumina next generation sequencing put a limelight over probable mechanism of shelf life extension of mulberry leaves by the application of phytosynthesized nanosilver solution. It was detected that genes associated with chloroplast and photosynthetic metabolism, detoxification of reactive oxygen and carbonyls species and innate immune response are mainly up-regulated in nanosilver preserved leaves. While loss of storage sucrose (sink metabolism), enhanced activity of senescence related hormonal mechanism, accumulation of xenobiotic compounds,

and development of osmotic stress inside tissue system was the probable reason for tissue deterioration in distilled water preserved leaves.

The outcome of the current dissertation work strongly recommends the use of green synthesized silver nanoparticle as an effective preservative solution in prolonging shelf life of mulberry leaves at post-harvest stage. Thus it becomes crucial to know the exact specification of synthesized nanosilver solution for its future application in the field of sericulture. The phytosynthesized nanosilver showed spectral band at ~441 nm, with high zeta stability and monodispersed particle distribution pattern. The biogenic nanosilver appeared crystalline in nature and spherical in shape with average particle size of ~14 nm and size distribution ranges from 12 – 40 nm. Thus current findings may help sericulture industry to regain the interest of landless farmers towards this wide field of application and may also help to overcome the long standing problem of farmers during monsoon season.