Abstract

Mulberry (*Morus alba* L.) is one of the most important cultivated crops in Indian sub-continent contributing in the direction of silk fibre production that carries economic significance worldwide. Present study deals with a challenge to resolve an age long difficulty of silkworm farmers which is feeding of silkworm larvae during monsoon season and a current arising dilemma related to landless silk farmers.

Silk industry was an agro-based labour intensive industry that can be sub-divided into two major sectors *viz.* cultivation of mulberry, for fresh leaves and rearing of silkworm. Cultivation of mulberry leaves for larval feeding is an outdoor practice, requires an open land, while rearing of silkworm larvae for cocoon is an indoor practice. Thus rearing exercise remains limited to those farmers bearing marginal to small scale lands. Landless farmers commonly drift from one garden to another or shift to urban areas in search of work leaving the conventional practice. Thus due to regular migration of traditional practitioners to urban areas, sericulture practice is shrinking day by day in India, putting an impact over economic status of the country. Beside this during monsoon, on feeding wet mulberry leaves, mortality rate of silkworm increases because of which production efficiency decreases, as a result silk farmer often incur economic loss during rainy season. Post-harvest mulberry leaf preservation by retaining natural condition of leaves using suitable preservative solution might help to overcome this problem.

Post-harvest preservation of mulberry leaves is a challenging factor as decrease in content of essential metabolites required for proper development of silk gland takes place. Decrease in concentration of chlorophyll, protein, sugar and increase in accumulation of ROS and associated free radicals takes place at post-harvest stages of preservation, putting negative impact on development of silkworm indicated by high mortality rate and low quality cocoon production. Biogenic silver nanoparticles were found to be effective in preserving leaves at post-harvest stage along with silver nitrate solution. Nanosilver at a concentration of 6 ppm was determined to be the least effective concentration for prolonging shelf life of mulberry leaves for at least 7 days at post-harvest stage, as revealed from appearance of physical texture along with retention of chlorophyll and other primary metabolites.

There are limitations of biogenic silver nanoparticles, as the dimension and geometry of nanoparticles produced through green synthesis (phytosynthesis) often varies greatly. To overcome this problem, process variation was conducted in which silver nanoparticles was synthesized by virtue of assessment of reducing efficacy of the plant extract at different concentrations of silver nitrate under diverse physical conditions *viz.* light intensity, pH and temperatures. Current study reports optimal physical condition for phytosynthesis of nanosilver as neutral pH (pH 7) and 25˚C under diffuse light (230 lux). Nanosilver formed by mixing 45 ml 10^{-3} M silver nitrate with 5 ml plant extract under process optimized condition showed monodispersed spherical shaped nanoparticles with size dimension range of 12 – 38 nm and zeta potential of +37.4 mV signifying stability. Beside this, synthesized nanoparticles were found to be highly stable under long storage condition as revealed by uniformity of SPR spectra. Nanosilver produced under optimized circumstance of pH 7 and at 25˚C under diffuse light was found to be bioactive as it showed potentiality to prolong the shelf life of mulberry leaves by seven days.

Solution of silver nanoparticle acts as an efficient preservative as it enhances the activity of enzymatic and non-enzymatic antioxidants thereby eliminating the harmful consequences of accumulated free radicals and ROS. The effectiveness of silver nanoparticle solution was found to be significantly higher than both silver nitrate (positive control) and distilled water (negative control), as greater retention of primary metabolites like pigments, proteins, and sugar necessary for healthy growth of silkworm larvae was noted. The impact of feeding nanosilver preserved leaves on silkworm larvae was observed to be significantly better than larvae fed with distilled water and silver nitrate preserved leaves. Larvae supplemented with nanosilver preserved leaves displayed growth rate, weight of cocoon and shell, effective rate of rearing almost equivalent to that of larvae supplemented with fresh leaves.

Beside these, the preservative effect of nanosilver solution was also determined by their potentiality to put off xylem blockage and inhibition of microbial propagation within preservative solution. Phytosynthesized silver nanoparticles displayed negative microbial count throughout the course of preservation as apparent from no colony-forming unit (CFU) till the final day of preservation, while conventional preservative silver nitrate displayed traces of CFU count on nutrient agar plate. Progressive increase in CFU count was recorded in distilled water set with increase in days of preservation. Beside these leaves preserved in solution of nanosilver showed almost negligible number of vascular blockage in petiole section approximately equivalent to the blockage pattern displayed by freshly harvested mulberry leaves. Whereas leaves preserved in control set (distilled water) showed large number of blocked vessels due to the deposition of macromolecules like protein, lignin and suberin. Nanosilver preserved leaves also displayed greater retention of membrane integrity than mulberry leaves preserved in both positive (silver nitrate) and negative (distilled water) control sets.

High-throughput RNA sequencing technology using Illumina platform has identified genes associated with chloroplast and photosynthetic metabolism; detoxifying reactive oxygen and carbonyls species; innate immune responses are mainly responsible for post-harvest shelf life extension in nanosilver preserved leaves. 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 negative control (distilled water preserved leaves). Real-time quantitative PCR validation of DEGs was in good agreement with RNA sequencing analysis, indicating reliability of the sequencing platform. RT-PCR analysis has validated the up-regulation of photosynthetic and stress management proteins in nanosilver preserved leaves.

Gel electrophoresis of mulberry leaf protein also reflects the preservative potentiality of nanosilver solution. Leaves preserved in solution of silver nanoparticle showed uniform protein banding pattern throughout preservation period. The protein banding pattern of leaves preserved in nanosilver solution appeared more prominent and invariable than the protein banding pattern of distilled water and silver nitrate sets. Protein banding pattern of silkworm larvae supplemented with nanosilver preserved leaves appeared roughly parallel to that of larvae supplemented with fresh leaves which also reflects effective preservative potential of nanosilver solution. Through isozyme profiling, superoxide dismutase and catalase activity were found to be active in both mulberry leaves preserved with nanosilver solution and silk gland of larvae supplemented with same preserved leaves. Isozyme profiling signifies presence of adequate defensive activity to shield against damage caused by ROS accumulation. OHR-LCMS profiled proteins through STRING analysis identified involvement of photosynthesis related and stress mitigating proteins, helping to avoid senescence thus enhancing shelf life. OHR-LCMS mulberry specific protein band (SDS-PAGE) profiling was in good agreement with the obtained results of NGS analysis.

Thus phytosynthesized (green synthesized) silver nanoparticles through prevention of microbial proliferation and vascular occultation, activated stress induced cellular defensive processes that helped in the retention of optimum primary metabolite content, including photosynthetic processes that directly promoting post-harvest shelf life extension up to 7 days. The physical texture, chemical profile and feeding preference of nanosilver preserved leaves was found to be almost equivalent to that of fresh mulberry leaves without any toxic effect, signifying the preservative potentiality of nanosilver solution over any other preservatives.