1. INTRODUCTION

Moraceae generally known as mulberry family or fig family is one of the commercially important family of flowering plants, cultivated across the globe. The family accompanied roughly 1100 species, representing 38 genera (Christenhusz and Byng 2016) and are globally distributed in the tropical and sub tropical regions and mostly confined in Asia and the Pacific Islands. Due to extremely diverse morphological articulation and habitat range, Moraceae is often considered as complex family. Characteristically all the species of this family produces milky sap. Datta (2002) reported that the genus Morus includes 68 species out of which one member is *Morus alba*, commonly known as white mulberry. *Morus alba* is one of the most economically important and cultivated genus of the family Moraceae. The genus *Morus alba* L. was placed by Bentham and Hooker (1862 – 1883) under the order Rosales in the family Moraceae. White mulberry is a small to medium sized tree, attaining maximum height up to 20 meter. The species is native to China (northern China) (Zhou et al. 2013) and widely cultivated in different geographical locations for fodder, silkworm rearing and timber production (Gupta 1993).

Silkworm (*Bombyx mori* L.) belongs to lepidopteron group of insect and is globally recognised as "Queen of textiles" for spinning out commercially important silk fibres (Soumya et al. 2017). Nagaraju (2002) stated that production of good quality silk fibres from silk gland of larvae utterly depends on larval internal metabolism and nutritional quality of mulberry leaves. The nutrient requirement of silk worm larvae is very sensitive as it requires proper balance diet of proteins, amino acids, carbohydrate, fatty acids, vitamins and various micro nutrients for normal growth and development of silk gland (Wani et al. 2018). Alebiosu et al. (2013) reported that ~70% of the total proteins present in silk fibre were directly contributed by mulberry leaves. The ability of silkworm larvae in converting protein present in mulberry leaf to silk protein depends on dietary efficiency, which in turn depends on quality of supplemented mulberry leaves (tenderness, thickness and tightness) they fed upon (Sharma et al. 2018). Low quality feeding resulted in low-grade, inferior quality small size cocoon and reduced number of eggs. Besides these low nutritive value also affects growth rate of larvae, body weight, movement, competitive ability longevity, and survival rate (Hossain et al. 2016).

The agro based silk industry is most often considered as one of the prime contributor to the rural lifeline of India as it generates employment among millions of rural and sub-rural people, improving economic status (Bukhari and Kour 2019). Based on division of labour, silk industry is divided into different sub-categories *viz.* mulberry cultivation, silkworm rearing including larval spinning, egg production, reeling and weaving and finally marketing (Bhat and Choure 2014). This agro-based labour intensive industry generates high employment among rural people (Kumar et al. 2013). More women participants were involved in sericulture practice in comparison to men. Women actively participate in silkworm rearing and management practices. Kasi (2013) reported that ~50% and 60% women participation were actively involved in the practice of mulberry cultivation and silkworm rearing respectively. Basically the rearing of silkworm consists of two important activities *viz*. cultivation of mulberry leaves and rearing of silkworm. Rearing is an indoor practice while cultivation of leaves is an outdoor practice which requires an open land. Thus rearing practice remains confined to those farmers having marginal to small scale lands (Sarkar et al. 2017). Land less farmers usually migrates from one garden to another or shift to urban areas in search of work leaving the conventional practice. Some farmers even purchase leaves from others gardens for performing the rearing practice (Sarkar et al. 2017). Regular purchasing of leaves and its carrying cost raises the overall cost of production, which has shifted the interest of traditional practitioners from this conventional field. Preservation by maintaining the leaf quality on purchasing the leaves once in a while may serve up as a way out to this problem by regaining the interest of farmers and their families to this wide field of application.

Another major challenging event faced by silk industry was rearing of silkworm larvae during rainy season. During rainy season, at the time of raining, rain water gets adsorbed in the surface of the leaf. Feeding of larvae with soggy leaves led to the development of larval disease consequence in high mortality rate, declining productivity. To overcome these crisis traditional practitioners for long time adopted the policy of leaf storage. The obstacle that is faced during storage is nutritional depletion and dehydration of leaves, resulting in mediocre quality cocoons. Post-harvest preservation of leaves in suitable preservative solution by retaining natural condition of leaves might assist to triumph over this existing problem.

Wilting, senescence, discolouration, high respiration rate, tissue browning, decay, vascular blockage and microbial growth are the major causes which checks postharvest shelf life extension and delimits the use of preservative solution. Microbial proliferation results in rapid senescence and breakdown of macromolecules by mounting ROS and free radical percentage (Merzlyak and Hendry 1994). Decolouration caused by chlorophyll degradation resulted in yellowing of leaves is the most conspicuous indicative happening of senescence (Merzlyak and Hendry 1994). In presence of light, leaf chloroplast is the main site for generation of ROS and during senescence disassembly of photosynthetic machinery causes disturbance of reducing equivalents of electron chain causing enrichment in ROS accumulation (Juvany et al. 2013). Unnecessary ROS accumulation causes damage at cellular level which is apparent by degradation of pigment, carbohydrates, proteins, lipids, and even nucleic acid (Das and Roychoudhury 2014). To overcome oxidative injury, tissue system of plants causes detoxification of surplus generated ROS by activating enzymatic and non-enzymatic enzymological activities (Sharma et al. 2012). ROS scavenging activity was triggered by the action of the antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPOX), ascorbate peroxidase (APX) and many others which works in a coordinated fashion transforming accumulated superoxide ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2) and finally to water (H_2O) (Ighodaro and Akinloye 2018; Apel and Hirt 2004). Glutathione, ascorbate, carotenoids are primarily involved in non-enzymatic enzymological activities. Glutathione participates in the generation of ascorbic acid which gets implicated in detoxifying superoxide inside chloroplast (Noctor and Foyer 1998), whereas carotenoids protects photosynthetic apparatus from damages caused by induction of stress (Racchi 2013). Phenolics are also classified as non-enzymatic antioxidants and are mainly concerned with phyto-based defence system by nullifying the toxic effect of generated free radicals (Rice-Evans et al. 1997).

With the on-set of stress, auto-activation of defensive pathways mediated by enzymatic and non-enzymatic molecules takes place. But these defensive activities decreases at post-harvest stage with increase in duration of preservation. Presence of bio-active elicitor or preservative may extend the shelf life by up-regulating the cellular defensive pathways. Silver nitrate is the most practiced and implicated silver salt as preservative in the field of horticultural crop, as they slow up microbial proliferation, preventing vascular occlusion (Elgimabi 2011). The impact of silver nitrate in elongation of vase life has been well documented and practiced in horticultural crop like rose (Hussen and Yassin 2013), tuberose (Anjum et al. 2001). Silver thiosulphate, another silver salt is frequently used as preservative for extending shelf life of horticultural crops. Silver nitrate and silver thiosulphate solutions are the most commonly used preservatives as they function as ethylene blocker, thus delaying the process of senescence (Subhashini et al. 2011). It has been reported that by acting as ethylene blocker both silver nitrate and silver thiosulphate delays senescence of flower and thereby prevents early abscission (Kofranek 1985; Knee 1992). Salts of silver ions were reported to extend the shelf life by maintaining the water column, as it also prevents the formation of microbial blockage in the xylem lumens (Ohkawa et al. 1999). It has been reported that there is feasible possibility of silver nitrate causing toxicity to living organism (Ratte 1999), putting a backward thrust towards its application. Nanosilver may serve as an alternate option as it has least toxic effect than any other forms of silver (Foldbjerg et al. 2009). Nanosilver solutions at low concentration might act as an effective preservative which not only prevents growth of microbial organisms (Naing et al. 2017), maintaining xylem integrity but also activates defensive enzymological activity (Hatami and Mansour 2014), maintaining pigment and vital metabolite concentration. Implementation of silver nanoparticle as preservatives has been reported to extend the shelf life of many flowering crops including *Chrysanthemum* (Carrillo-López et al. 2016), *Dianthus* (Solgi 2018), Gerbera (Kazemi and Ameri, 2012) and tulip (Byczyńska 2017). However almost all of the preservative aspects of nanosilver and silver salts have been reported for extending vase or shelf life of commercially important flowering twigs, almost no report was obtained that describes their potentiality towards prolonging shelf life of cultivated leaves.

Silver was one of the most universally practiced metal nanoparticles due to its nontoxic, harmless influence on humans and bioactivity at low concentrations (Singh et al. 2015). Historical records suggest greater payback of silver than the hazardous impact, due to its therapeutic and medical benefits (Alexander 2009). Silver nanoparticles have broad spectrum application as antimicrobial agents in preventing infections. Beside these, silver nanoparticles bear diverse application in the fields of catalysis, photonics, electronics, and optics (Bhaduria et al. 2013). Due to the capability of nanosilver to generate high amount of free-radicals, it was considered as one of the best metallic nanoparticles suitable for biological applications as antibacterial, antimicrobial, antifungal and antiviral agents (Kim et al. 2007).

Different techniques have been adopted by different workers of the globe concerned with the synthesis of metal nanoparticles *viz*. chemical reduction, photochemical reduction, microwave irradiation, micro-emulsion, gamma-ray irradiation, laser ablation, electrochemical reduction, and many others (Chauhan et al. 2019; Srikar et al. 2016). Application of these traditional methods have countless drawbacks often results in creating hazardous impact over environment which includes application of flammable organic solvents and harmful chemicals, high toxicity, capital intensive as requisite expensive instruments, high energy consumption, huge amount of waste generation and difficulties associated with purification (Gnanavel et al. 2017; Rai et al. 2009). Beside this, nanosilver synthesized by chemical methods was inappropriate for medical application as there resides likelihood of toxic effect of the chemicals used as reducing and capping agent (Samari et al. 2018). So in concern to global safety, necessitate for development of novel substitute methods for synthesis of nanoparticles with controlled shape and size having energy-efficient, eco-friendly and non-toxic properties. Use of biological materials under the kingdom Plantae could be efficiently explored for the development of simple, rapid, stable, eco-friendly, cost-effective and hygienic nanoparticles, thus opening a new path in the form of green chemistry commonly called as biogenic synthesis or green synthesis (Elemike et al. 2017).

Green chemistry utilizes bacteria, algae, fungi and plants that include bryophyte, pteridophyte, gymnosperm and angiosperm (Singh 2019; Srikar et al. 2016) for phytosynthesis of metallic nanoparticles. Among the diverse groups of biological organisms, green plants gains added advantage over others as they drastically reduces the reaction and reduction time from several days to few minutes that was required by microbes; besides this they are cost effective as they do not require high energy, high pressure and toxic chemicals and can be easily scaled up for large-scale production at industrial level (Yang et al. 2014; Kaushik et al 2010). Besides these, wide-ranging versatility among plants serves as a affluent source of various phytochemicals including aldehydes, ketones, amides, flavones, terpenoids, carboxylic acids, phenols and ascorbic acids that carries the significant potentiality for reducing metal salts into metal nanoparticles (Singh et al. 2018). Phytosynthesis of silver nanoparticles has been reported using the extract of *Wrightia tinctoria* (Rajathi and Sridhar 2013), *Withania somnifera* (Raut et al. 2014), *Tephrosia purpurea* (Ajitha et al. 2014), *Abutilon indicum* (Kumar et al. 2015), *Ziziphora tenuior* (Sadeghi and Gholamhoseinpoor 2015), *Salvia hispanica* (Joshi et al. 2019), *Priva cordifolia* (Ananda et al. 2019) and *Flemingia wightiana* (Reddy et al. 2020). Some study also reports the use of naturally biodegradable plant components *viz.* polysaccharides, vitamins, enzymes, biopolymers and latex for biosynthesis of silver nanoparticles (Shukla and Iravani 2017; Talekar et al. 2016). Current study reports biosynthesis of nanosilver using *Morus alba* leaf extract due to its pre-established medicinal and nutraceutical properties making it non-toxic to mankind (Li 1998).

Morus alba (Mulberry), plant which bears not only monetary value but also having medicinal importance. Wild and cultivated species of mulberry are distributed across India (Tikader and Vijayan 2010), making their easy availability. Global value of mulberry lies in the exploitation of its leaves for the feeding of monophagous insect *Bombyx mori*. The potentiality of mulberry also lies in its anti-diabetic (Lee et al. 2002), antioxidant (Wattanapitayakul et al. 2005; Oh et al. 2002), antimicrobial (Sohn et al. 2004), anticancerous (Kofujita et at. 2004), neuroprotective (Niidome et al. 2007), and hepatoprotective (Hogade et al. 2010) activity. Because of its significant medicinal importance, mulberry leaves along with its root and stem are consumed in different parts of world directly as tea (Thaipitakwonga et al. 2018; Wilson and Islam 2015). Current work is based on the hypothesis that synthesized nanoparticles will bear effective bioactive antimicrobial and antioxidant activity.

Earlier researchers have performed green synthesis of silver nanoparticles using dry extract of mulberry leaves, which was either dried in direct sun light (Awwad and Salem 2012) or shade dried (Singh et al. 2018; Akbal et al. 2016). Oxidative change in phytochemical constituents of leaves may occur during the process of drying, which might put significant impact over the process of reduction during nano formation. To avoid occurrence of any such unwanted oxidative reconstruction of phyto-constituents, current study was designed to use aqueous decoction of fresh mulberry leaves through refluxing for the formation of silver nanoparticles.

The foremost disadvantage of green synthesis of nanoparticles resides with uncontrolled grip over size and shape of biosynthesizing nanoparticles. In green chemistry phytochemical constituents present in plant extract directly acts as reducing and capping agent, so it was extremely difficult to predict their structure, approach of interaction and nature of alliance at the time of reaction, often leads to the formation of large and variable size particles with diverse morphology putting a check on their utilization at commercial level (Gour and Jain 2019). As nucleation and growth of crystals take place at the identical point in time, so it was incredibly difficult to control particles size distribution (LaMer and Dinegar 1950). Thus from bioactivity point of view, size of green synthesized silver nanoparticles was very crucial thereby putting a demand for controlled synthesis leading to the development of novel size nanosilver. It has been proposed that control over size and shape of nanoparticles can be achieved by adjusting pH and temperature of the reaction medium that brings changes to the nature of the reducing agent (Ibarra-Sánchez et al. 2015). Beside this for increasing long term stability and overall yield percentage of biogenic silver nanoparticles, process optimization of physical parameters *viz.* pH, temperature and light intensity was the elementary prerequisite (Birla et al. 2013). It has been reported that difference in nanoparticles size can be achieved by altering the condition like time, pH, temperature, reactants concentration and ratio of plant extract to metallic salt of the standard process (Liu et al. 2017). Gardea-Torresdey at al. (2003) reported control over shape and size of the nanoparticles by altering the pH that was biosynthesized using alfalfa sprouts. Fayaz et al. (2009) during nanoparticle synthesis reported change in size of particles with increase and decrease in temperature from optimum range. Increase in temperature enhances the tempo of reaction, as well as reduction rate and thereby putting impact over distribution of particle size (Khalil et al. 2014).

Current dissertation work was carried out considering the hypothesis that green synthesized nanosilver will bear the ability to prolong the shelf life of mulberry cultivars at postharvest stage. In the current study five attempts were made, first bioactive green synthesis of silver nanoparticles; secondly detection of ideal condition of biosynthesis of nanoparticles through process variation; thirdly to investigate the effect of nanosilver as preservative solution, in prolonging shelf life of mulberry leaves by retaining valuable metabolite concentration; fourth assessment of comparative aspects among different preservative solutions; and lastly whether there is any adverse effect of feeding preserved mulberry leaves on rearing system of silkworm larvae.

Based on the above hypothesis the objective of current dissertation works are prescribed below:

a. Green synthesis of silver nanoparticles using mulberry leaf extract, its characterization, process variation and stability assessment

b. Antioxidant and antimicrobial activity assessment of prepared nanosilver

c. Application of silver nanoparticles and salts of silver ion in preservation of mulberry leaves their biochemical and phytochemical characterization

d. Assessment of stress accumulation and alternation in free radical scavenging activity of preserved mulberry leaves

e. Determining the changes in antioxidant enzymological characteristics of mulberry leaves after preservation

f. Determining the impact of feeding mulberry leaves preserved with nanosilver and salts of silver ion on larval and cocoon parameters

g. Analysis of gel electrophoresis and isozyme patterns by polyacrylamide gel electrophoresis of preserved mulberry leaves and silkworm larvae fed with preserved leaves

h. Comparative transcriptome analysis of nanosilver preserved leaves with respect to leaves preserved in distilled water.