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Research Article

EVALUATING THE EFFECT OF SILVER IONS IN PRESERVATION OF MULBERRY LEAVES AND ITS IMPACT OVER SILK WORM REARING SYSTEM

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ABSTRACT

Present study was done to evaluate the effect of feeding preserved mulberry leaves over silk worm rearing system. After harvesting, leaves were preserved for seven days with two different silver salts at three different concentrations - silver nitrate (10, 50, 100 ppm) and silver thiosulphate (50, 100, 200 µM). Fresh leaves (0 day preservation) were taken as control. After seven days of preservation the leaves were given to fifth instar silk worm larvae for feeding. The result suggests that among the two silver salts, silver nitrate served as more effective preservative for protecting mulberry leaves than silver thiosulphate which was confirmed by analyzing the biochemical, phytochemical and antioxidant attributes of preserved leaves compared with control. Rearing parameters indicate that larvae fed with leaves preserved in 10 ppm silver nitrate solution showed best result while mortality rate increased among larvae when fed with leaves preserved with silver thiosulphate solution.

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INTRODUCTION

The principal and sole food for *Bombyx mori* (Silk worm) is *Morus alba*, placed under the family Moraceae by Bentham and Hooker. The complete growth and development of silk worms depends on nutritional level of mulberry leaves they feed upon (Radjabi *et al*., 2010). The nutritional level of mulberry leaves varies from one cultivar to another, besides these climatic factors and geographical location also play important role for assimilation of various nutrients (Ito, 1978). Deficiency in nutrient level in mulberry leaves will directly hamper the growth and development of silk worm larvae as it will reduce primary and secondary metabolite content of leaves leading to the generation of oxidative stress.

One of the prime problem that is faced by silk industry since long is the feeding of larvae during rainy season because feeding wet leaves is harmful for the entire rearing process, as it may lead to death of the larvae. One of the possible solution to overcome this problem will be to collect the bulk quantity of leaves during dry days of rainy season and preserved in such a way that leaf quality retains almost to that extent of fresh leaves.

The major challenges faced during leaf preservation are wilting and yellowing of leaves, as well as blockage of xylem due to microbial contamination; use of preservative solutions might be an effective way to overcome all these hurdles. Silver nitrate (SN) and silver thiosulphate (STS) solutions are the most common preservatives as they act as ethylene blocker, thus delaying senescence (Subhashini *et al.,* 2011). By acting as ethylene blocker both SN and STS delays senescence of flower and thereby prevents abscission (Kofrenek, 1985; Knee, 1992). Salts of silver ion extend the shelf life by maintaining the water column, as it prevents the formation of microbial blockage in the xylem vessels (Ohkawa *et al.,* 1999). Butt (2005) observed that 150 ppm of SN extends the vas life of *Rosa hybrida* by 4 days. Sharma and Bhardwaj (2015) stated that application of SN as preservative increased the longevity of cut flowers by acting as ethylene biosynthetic blocker and also by inhibiting microbial growth. Application of STS extends the vas life of cut rose flower stem up to 10 days (Reid *et al.,* 1980). Ten minute pulsing of STS increases the post harvest shelf life of cut carnation flowers (Dimalla and Staden, 1980). Liao (2000) observed that production of ethylene in cut rose decreases at post harvest stage if treatment of STS was given. Although lots of work has already been accomplished on SN and STS as ACC synthase blocker, but most of the work remains restricted in preservation of economically important flowering twigs and almost no work was done for evaluating the effect of silver ions

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in preservation of economically important leaves at post harvest stage along with senescence retardation.

As silver ions have already proved their potential for retarding senescence in most of the experimental crops, it might be expected that it will also play positive role in extending the shelf life of economically important mulberry leaves at postharvest stage. The aim of our current study is to evaluate whether salts of silver ions (SN and STS) could bear the capacity to extent the shelf life of mulberry leaves at post harvest stage; besides these the effect of feeding these preserved leaves over silk worm and cocoon parameters will also be evaluated for determining whether silver impregnated preserved leaves put any adverse effect over larval growth and in cocoon formation.

MATERIAL AND METHOD

Plant sample collection

From Matigara Sericulture Farm, Siliguri, West Bengal, India mature, fresh and disease free leaves of S1 mulberry cultivars were collected during August to October, 2017. The area was located at 26°70’40”N and 88°35’37”E.

Preservation of leaves

Leaves were preserved in small superior quality Borosil made glass beakers. Before preserving leaves, fine oblique section under water was made to the petioles of the leaves so that water column remains intact. The glasses containing the leaves were inserted inside zipper bag for retaining moisture content with small perforations for gaseous exchange.

Leaves were preserved with distilled water, silver nitrate solution (10, 50 and 100 ppm) and silver thiosulfate solution (50, 100 and 200 µM) for seven days. At the end of seventh day different biochemical, phenylpropanoid and antioxidant screening were carried out to predict the differences in respect to fresh leaves, along with this fifth inster larval feeding was also conducted taking fresh leaf as control to evaluate the difference in feeding preserved leaves and fresh leaves over larval parameters.

Biochemical screening

Estimation of chlorophyll content

Chlorophyll content was measured following Arnon method (1949). Preserved leaves were homogenized in 80% acetone and the green filtrate was collected and the absorbance was measured at 663 nm and 645 nm for calculating the amount of chlorophyll present in the extract.

Estimation of protein content

Lowry’s method (1951) was used for estimating total protein content. To 1 ml extract 5 ml alkaline copper solution and 0.5 ml Folin-ciocalteu reagent was added and mixed well. The intensity of the blue coloured complex formed was measure at 660 nm. Total protein content was estimated by using standard curve prepared from BSA.

Estimation of total soluble sugar content

Total soluble sugar was estimated following the method prescribed by Anthrone (Thimmaiah, 2004). To 1 ml of extract 4 ml anthrone reagent was added and incubated for 10 min at 100º C. The mixture was then brought to room temperature and absorbance was taken at 620 nm. Total carbohydrate content was calculated by using sucrose as standard.

Estimation of reducing sugar content

DNSA method (Sadasivam and Manickam, 1998) was used for estimating reducing sugar content. To 1 ml 3, 5 Dinitrosalicylic acid (DNSA), sample extract was added and boiled for 5 min. The coloured product that was developed, to it 40% Rochelle salt solution was added, mixed well and the mixture was allowed to cool. Absorbance was taken at 510 nm and reducing sugar content was calculated using standard curve prepared from glucose.

Estimation of total phenol content

Method prescribed by Kadam *et al.* (2013) was followed for estimating total phenol content with slight modifications. For estimating, 95% ethanol, 5 ml distilled water, 50% Folin ciocalteau reagent and 5% Na_2CO_3 was added to 1 ml sample extract and incubated for 1 hour. Absorbance was measured at 725 nm and phenol content was estimated using gallic acid standard curve.

Estimation of orthodihydric phenol

Method prescribed by Mahadeven and Sridhar (1986) was followed for estimating orthodihydric phenol. To 0.5 ml methanolic extract of the sample, 0.5 ml Arnow’s reagent, 5 ml distilled water and 1 ml 1 (N) NaOH was added. Absorbance of the developed pink colour was taken at 515 nm and standard curve was prepared using catechol as standard.

Estimation of flavonoid content

Flavonoid content was estimated following the method of Atanassova *et al.* (2011). To 0.5 ml extract 4 ml distilled water, 5% $NaNO_2$, 10% $AlCl_3$ and 2 ml 1 (M) NaOH was added leading to the development of pink colour, absorption of which was taken at 510 nm. Flavonoid content was estimated using standard curve prepared from quercetin.

Study of antioxidant activities

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

DPPH radical scavenging activity was measured according to Sidduraju *et al.* (2002). For estimation, 2 ml DPPH was added to 0.2 ml methanol extract and absorbance was measured at 517 nm. Scavenging activity was measured as percent inhibition using the following equation:

% inhibition = $[(A_0 - A_1)/A_0]$ 100%

Where A_0 is the absorbance of the control and $\mathbf{A_1}$ is the absorbance of the sample. Antioxidant activity was expressed as concentration where 50% reduction in free radical takes place referred to as IC_{50} value.

$ABTS^+$ (2, 2-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid) scavenging assay

Method of Li *et al.* (2009) was followed for $ABTS^+$ scavenging activity. To 1 ml methanolic extract 2 ml ABTS was added and incubated for 10 min. Absorbance was measured at 734 nm and percent inhibition and antioxidant activity was measured in the same way as stated above.

Superoxide radical (SO) scavenging assay

Method prescribed by Fu *et al.* (2010) was used for estimating superoxide scavenging activity. One ml nitroblue tetrazolium chloride, 1 ml nicotinamide adenine dinucleotide and 10 µl phenazine methosulphate were added to the methanol extract and incubated for 30 min. Absorbance was measured at 560 nm and percent inhibition and antioxidant activity was measured using the same formula as stated above.

Nitric oxide (NO) scavenging assay

Method prescribed by Marcocci *et al.* (1994) was used to measure nitric oxide scavenging activity. 20 mM sodium nitroprusside, 0.5 ml phosphate buffer and 3 ml Griess reagent was mixed with 0.5 ml extract and incubated for 30 min. Absorbance was measured at 540 nm and percent inhibition and antioxidant activity was measured in the same way as stated above.

Feeding experiment and rearing data collection

The overall rearing process was conducted under laboratory condition following the standard process of Krishnaswami *et al.* (1978). For rearing purpose healthy and disease free fifth instar larvae (Nistari) were collected from sericulture farm, Matigara, Siliguri. The larvae were distributed uniformly and randomly in bamboo made trays so that each tray contains ten larvae. For feeding, larvae are supplemented with S1 leaves. In one set freshly collected S1 leaves were given for feeding which serves as control, while in other sets leaves preserved with distilled water, silver nitrate and silver thiosulfate solution were given for feeding. During rearing process larval weight was recorded at regular interval. Besides this, mortality rate was also measured. When the larvae started spinning they were left undisturbed. At the end of the trial growth index (GI), single cocoon weight (SCW), single shell weight (SSW), shell ratio (SR) (%), effective rearing rate (ERR) (%) and mortality rate (MR) (%) were calculated using following formulas:

$$GI = \frac{\text{Final weight of larvae (gm)} - \text{Initial weight of larvae (gm)}}{\text{Initial weight of larvae (gm)}}$$

$$WCS = \frac{\text{Weight of 10 male cocoon (gm)} + \text{Weight of 10 female cocoon (gm)}}{\text{Total number of cocoon (20)}}$$

$$SSW = \frac{\text{Weight of 10 male shell (gm)} + \text{Weight of 10 female shell (gm)}}{\text{Total number of shell (20)}}$$

$$SR\ (\%) = \frac{\text{Single shell weight (gm)}}{\text{Single cocoon weight (gm)}} \times 100$$

$$ERR\ (\%) = \frac{\text{Total number of cocoon harvested}}{\text{Total number of larvae brushed}} \times 100$$

$$MR\ (\%) = \frac{\text{Number of death larvae}}{\text{Total number of larvae}} \times 100$$

Statistical analysis

Results were expressed as Mean ± SEM where n = 3 for leaf parameters and 10 for larval parameters. Difference between different preservatives are indicated with different letters (a, b, c, etc.) differ significantly at $p \leq 0.05$ by Duncan's Multiple Range Test (DMRT). The data were subjected to analysis of variance (ANOVA) to determine the level of significance of different attributes under study. Similarity Dendrogram was prepared using XLSTAT 2017 Software for determining the effect of preservatives by cluster analysis. Relationship between different attributes, stages of preservation and rearing parameters were studied by preparing Heat map using R-software (version 3.4.0).

RESULT AND DISCUSSION

For producing good and superior quality silk, silk worm larvae mostly depends on quality of nutrient they get by feeding upon mulberry leaves. Healthy and disease free larvae have a greater chance of producing high quality silk and for a larvae to become healthy, nutrient balance is important which they obtained by feeding upon mulberry leaves, so indirectly nutrient balance inside leaf is important for producing good quality of silk. Murthy *et al.* (2013) stated that alteration of metabolic activity of silk worm larvae take place if imbalance occurs in leaf nutrient content. So retention and maintaining nutrient content within mulberry leaf is the prime factor for producing good quality silk.

On assessing the physical condition of leaves it was revealed that leaves preserved in 10 ppm SN solution resembles almost same to that of fresh leaves. Figure 1 represents the physical appearance of leaves after 7 days of preservation in different concentration of SN and STS, the order of preservation on the basis of visual observation was as follows: fresh > 10 ppm SN > 50 ppm SN > 50 µM STS > 100 ppm SN > 100 µM STS > 200 µM STS > d.H_2O.

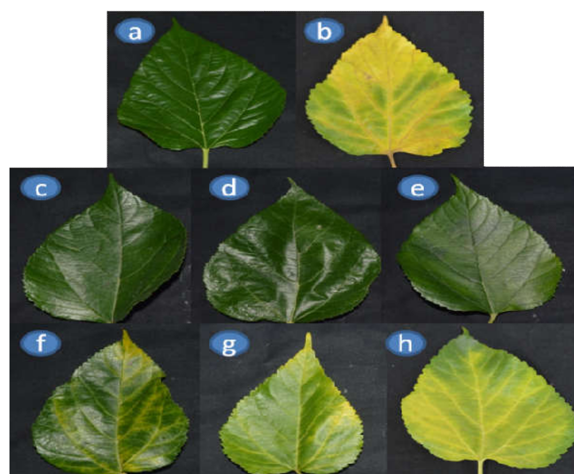

Fig 1 Morphological condition of leaves after seven days of preservation. a: fresh leaves; b: distilled water preserve leaf; c - e: 10, 50, 100 ppm SN preserved leaf respectively; f - h: 50, 100, 200 µM STS preserved leaf respectively.

For determining the nutrient condition of preserved leaves in comparison to fresh leaves different biochemical analyses were performed (Table 1). Chlorophyll is essential as it takes part in photosynthesis and thereby synthesizing carbohydrate necessary for proper development of leaves. The photosynthetic efficiency and quality of foliage can be determined by quantifying chlorophyll content (Murthy *et al.*, 2013). Current study revealed that in comparison with control, retardation of chlorophyll content was least in leaves preserved with 10 ppm SN solution (2%) while maximum decline in chlorophyll content was noticed in leaves preserved with distilled water (80%). From the result obtained it can be stated that SN as an anti-senescence agent prevents senescence and might also retain total chlorophyll content.

Table 1 Effect of preservation on different biochemical attributes

TREATMENT	Total Chlorophyll (µg/mg FWT)	Total PROTEIN (µg/mg FWT)	Total Soluble Sugar (µg/mg FWT)	Reducing Sugar (µg/mg FWT)	Proline (µg/mg FWT)
0 d - Control	1.95±0.08a	53.07±0.96^{a}	40.65±0.41^{a}	5.73±0.12^{a}	0.59±0.32^{a}
7 d - Control	0.39±0.03^{d}	16.33±1.10^{d}	20.21±1.35^{d}	1.99±0.02^{f}	4.39±0.03^{e}
7d - SN 10 ppm	1.93±0.03^{a}	52.51±0.70^{a}	40.07±0.42^{a}	5.68±0.10^{a}	1.02±0.01^{a}
7d - SN 50 ppm	1.76±0.18^{ab}	48.47±1.34^{a}	38.42±0.67^{a}	4.93±0.03^{b}	2.24±0.03^{b}
7d - SN 100 ppm	1.45±0.09^{bc}	36.93±1.72^{b}	33.38±0.47^{b}	4.48±0.02^{c}	2.59±0.19^{bc}
7d - STS 50 µM	1.50±0.17^{abc}	32.93±1.74^{b}	31.46±0.41^{b}	4.45±0.03^{c}	2.44±0.33^{bc}
7d - STS 100 µM	1.11±0.13^{c}	23.70±0.91^{c}	25.66±0.92^{c}	3.88±0.05^{d}	3.22±0.58^{cd}
7d - STS 200 µM	0.61±0.11^{d}	15.71±1.45^{d}	21.49±0.44^{d}	2.54±0.05^{e}	3.95±0.89^{de}

Results are expressed as Mean ± SEM of triplicate determinations. Values with different letters (a, b, c, etc.) differ significantly (p≤0.05) by Duncan's Multiple Range Test (DMRT).

For proper growth of larvae and its silk gland, protein content present in mulberry varieties might play an important role (Bongale and Chaluvachari, 1995). In present study it was found that fresh S1 leaves contain 53.07µg/mg FWT protein; on assessing the protein content of preserved leaves it was observed that leaves preserved in 10 ppm and 50 ppm SN solution retains the protein content almost similar to that of fresh leaves while decline in protein content was noted in all other solutions with maximum decline in 200 µM STS solution which was approximately 70%. Jyothi *et al.* (2014) stated that larva that belongs to Lepidopteron group mainly depends upon leaf protein for proper nutrition, beside this 70% of total protein present in raw silk comes from leaf protein. SN by acting as ethylene inhibitor prevents senescence and thereby prevents degradation of proteins, beside this as an antimicrobial agent SN may prevents protein utilization caused by microbes.

Besides protein, carbohydrate serves as an energy source inside larval body which directly promotes growth and development of silk worm and ultimately to the quality and quantity of silk (Kumar and Michael, 2012). Leaves preserved with 10 ppm and 50 ppm SN solution retains carbohydrate content approximately by 98% and 94% respectively in comparison with fresh leaves, while in all other preservatives solution retention of carbohydrate content is less that 80% with least retention in leaves preserved in distilled water for 7 days which was approximately 49%. Thus it can be speculated that as an antimicrobial agent SN prevent blockage of vascular tissue caused by microbes and due to which photo-assimilates (carbohydrates) can be transported easily to different tissues maintaining sugar level.

Proline content acts as stress indicator, Kala and Godara (2011) stated that fresh and healthy mulberry leaves bear minimum proline content which gradually enhances with stress. On assessing the proline content in present study it was observed that in comparison with control proline content increases in all the preservative sets with maximum increase in leaves preserved with distilled water and 200 µM STS solution which was approximately four fold indicating the osmolyte requirement under stressed condition while least increase was observed in 10 ppm SN solution pointing its fresh and healthy nature.

On assessing the poly-phenol (phenol, ortho-dihydric phenol and flavonol) content, it was observed that during preservation increase in poly-phenol content was observed in leaves preserved with all concentrations of SN along with 50 and 100 µM concentration of STS solution (Table 2).

Table 2 Effect of preservation on different phenyl propanoid attributes

Treatment	Phenol (µg/mg FWT)	Orthodihydric Phenol (µg/mg FWT)	Flavanol (µg/mg FWT)
0 d - Control	7.98±0.07^{d}	1.77±0.09d^{e}	3.93±0.06^{e}
7 d - Control	7.60±0.10^{d}	1.30±0.03^{e}	3.42±0.12^{f}
7d - SN 10 ppm	12.15±0.06^{a}	3.41±0.12^{a}	6.79±0.16^{a}
7d - SN 50 ppm	11.86±0.33^{a}	2.80±0.27^{b}	5.12±0.11^{c}
7d - SN 100 ppm	10.49±0.16^{b}	2.64±0.11^{bc}	5.80±0.16^{b}
7d - STS 50 µM	9.45±0.11^{c}	2.11±0.12^{cd}	4.53±0.13^{d}
7d - STS 100 µM	9.09±0.10^{c}	2.03±0.03^{d}	4.44±0.11^{d}
7d - STS 200 µM	7.61±0.08^{d}	1.44±0.03^{e}	3.25±0.11^{f}

Results are expressed as Mean ± SEM of triplicate determinations. Values with different letters (a, b, c, etc.) differ significantly (p≤0.05) by Duncan's Multiple Range Test (DMRT).

The increasing order of poly-phenol content in leaves during preservation is as follows d.H_2O < 200 µM STS < Fresh < 100 µM STS< 50 µM STS10< ppm SN< 50 ppm SN<10 ppm SN. Phenols act as an effective hydrogen donor and it plays role in quenching singlet oxygen (Gupta and Mandal, 2015). Kahkonen *et al.* (1999) stated that many biologically active compounds are present in phenols. Ghasemzadeh and Ghasemzadeh (2011) reported that flavonoids take part in defence system by acting as reducing agent, free radical scavenger and quencher of singlet oxygen. Thus it may be stated that during preservation increase in phenolics helps to nullify the ROS produced due to stress and thereby increasing shelf life.

Free radical scavenging activity of fresh and preserved leaves in different preservative solutions were measured in terms of DPPH, ABTS, SO and NO scavenging activity and were expressed in terms of IC_{50} value (Fig. 2).

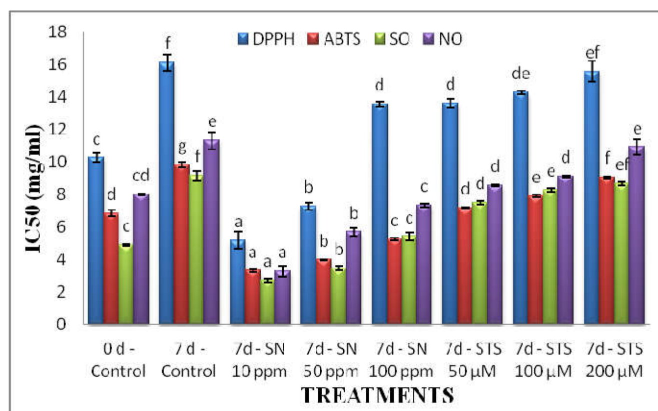

Fig 2 Bar graph representing free radical scavenging activity of preserved leaves

Free radical scavenging activity increases during preservation in leaves preserved with 10 ppm and 50 ppm SN solution which was revealed by lower IC_{50} value than fresh leaves, while leaves preserved in remaining solutions showed less

efficiency to nullify free radicals. Antioxidants help to protect the cells from damage caused by free radicals during stress condition (Badakhshan *et al.,* 2012). Free radical scavenging activity mainly depends upon structure of the compound to be neutralized and hydrogen donating availability of antioxidant compound (Shimada *et al.*, 1992; Fukumoto and Mazza, 2000). From the result obtained it may be stated that lower concentration of SN solution preserves the leaves in such a way that the leaves retain the power to nullify free radicals formed during prolonged preservation.

For evaluating effect of feeding preserved leaves over larval and cocoon parameters, leaves preserved with distilled water, 10 ppm, 50 ppm, 100 ppm SN solution and 50 µM, 100 µM, 200 µM STS solution for 7 days were used to feed upon fifth instar larvae (Nistari) for 6 days after which the larvae started spinning. Table 3 represents different attributes that were evaluated to screen out the effect of feeding preserved leaves over larval growth and cocoon parameters. Murugan *et al.* (1998) stated that growth and development of larvae depends upon various factors that play their role both inside and outside the larval body.

concentration, indicating that leaf quality decreases when STS was used as preservative. The descending order of feeding preserved leaves over larval parameters can be listed as follows: fresh > 10 ppm SN >50 ppm SN >100 ppm SN > 50 µM STS >100 µM STS >200 µM STS > distilled water.

To determine the degree of significance between different attributes under study ANOVA analysis was preformed. From ANOVA analysis (Table 4) it can be said that leaves preserved in different preservatives bear significant impact on all the biochemical, phytochemical, antioxidant and rearing parameters under study at p<0.01 level. For determining the correlation between different preservative solutions used with respect to their performance in preserving the mulberry leaves, agglomerative hierarchical clustering (AHC) was performed (Fig. 3) using Pearson similarity dendrogram. AHC result suggests that 10 ppm SN preserved leaves showed highest resemblance with fresh leaves in terms of overall performance, while distilled water preserved leaves grouped with 200 µM STS preserved leaves indicating their resemblance with respect to their performance.

Table 3 Effect of feeding preserve leaves over rearing parameters

TREATMENT	Growth Index	Single Cocoon Weight (gm)	Single Shell Weight (gm)	Shell Ratio (%)	Effective Rate of Rearing (%)	Mortality Rate (%)
0 d - Control	1.45±0.02[a]	1.04±0.01[a]	0.21±0.02[a]	20.22±0.07[a]	86.67±3.33[a]	13.33±3.33[a]
7 d - Control	0.33±0.01[c]	0.55±0.10[b]	0.05±0.01[c]	8.14±0.07[g]	16.67±3.33[g]	86.67±3.33[f]
7d - SN 10 ppm	1.44±0.02[a]	1.06±0.03[a]	0.21±0.03[a]	19.89±0.12[b]	86.67±3.33[ab]	13.33±3.33[a]
7d - SN 50 ppm	1.43±0.02[a]	1.03±0.01[a]	0.19±0.02[a]	18.73±0.13[c]	76.67±3.33[ac]	26.67±3.33[b]
7d - SN 100 ppm	1.30±0.10[a]	1.03±0.02[a]	0.16±0.02[ab]	15.14±0.02[d]	66.67±3.33[d]	36.67±3.33[c]
7d - STS 50 µM	1.26±0.11[a]	1.00±0.01[a]	0.15±0.02[ab]	14.96±0.09[d]	56.67±8.82[e]	46.67±8.82[d]
7d - STS 100 µM	0.76±0.06[b]	0.40±0.03[bc]	0.09±0.01[bc]	14.24±0.01[e]	36.67±8.82[f]	60.00±11.55[e]
7d - STS 200 µM	0.40±0.04[c]	0.35±0.02[c]	0.04±0.01[c]	12.47±0.05[f]	20.00±5.77[g]	80.00±5.77[f]

Results are expressed as Mean ± SEM, n=10. Values with different letters (a, b, c, etc.) differ significantly (p≤0.05) by Duncan's Multiple Range Test (DMRT)

Table 4 ANOVA analysis representing the significance level among different parameters under study

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Treatments	38385.59	17	2257.976	20.60945	1.55E-28	1.704427
Parameters	13804.59	126	109.5602			
Total	52190.17	143				

Significant at p<0.01 level

Quality assessment of cocoon crop depends on important larval and cocoon parameters such as effective rate of rearing, single cocoon weight, single shell weight and shell ratio (Malik *et al.,* 2006), thus by studying this parameters the effect of feeding preserved leaves can be evaluated. GI of larvae fed with leaves preserved with distilled water for 7 days decreased by 77.24% in comparison to larvae fed with fresh leaves. Decrease in GI puts severe effect on SCW, SSW, SR (%) and ERR (%); besides these in comparison to control mortality rate increased by approximately 73%, indicating that toxicity level rises abruptly in leaves preserved with distilled water which puts significant impact on larval growth and cocoon parameters. When larvae were fed with leaves preserved in SN and STS solution, it was found that larvae fed with leaves preserved in 10 ppm SN gives the best result almost similar to that of feeding fresh leaves, which reveals that SN at low concentration acts as effective preservative by retaining the leaf quality and does not allow the formation of any toxic product which can put negative impact over larval growth. When larvae were fed with leaves preserved in STS solution, it was observed that decrease in larval and cocoon parameters and gradual increase in mortality rate takes place with increasing

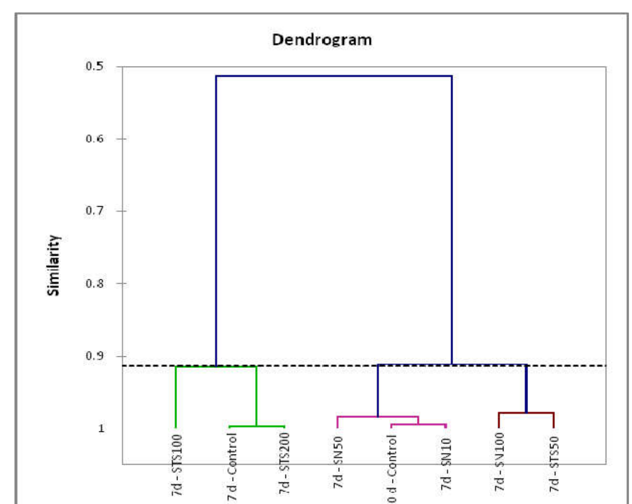

Fig 3 Dendrogram representing similarity clusters of different preservative solutions with respect to overall parameters

Heat map was prepared to correlate and study different attributes both by graphical illustration and by hierarchical

cluster trees on the vertical and horizontal axes (Fig. 4). In the present study different preservation stages were placed on the vertical axis, while biochemical, phytochemical, antioxidant and rearing parameters were placed on horizontal axis.

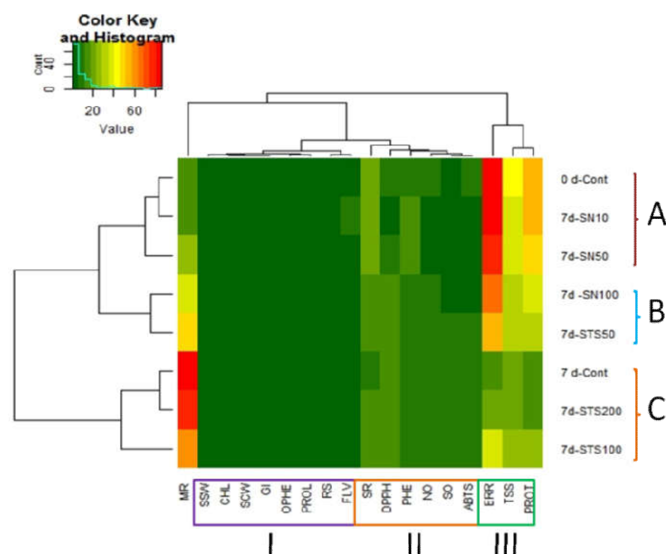

Fig 4 Heat map analysis representing cluster relationship between different preservative solutions with different parameters under study

CHL = Total chlorophyll, PROT = Protein, TS = Total sugar, RS = Reducing sugar, PHE = Phenol, OPHE = Orthodihydric phenol, FLV = Flavonol, DPPH = 2, 2-diphenyl-1-picrylhydrazyl, ABTS = 2, 2-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid, SO = Super oxide, NO = Nitric oxide, GI = Growth index, SCW = Single cocoon weight, SSW = Single shell weight, SR = Shell ratio, ERR = Effective rate of rearing, MR = Mortality rate

On the vertical axis fresh leaves, 7 day preserved leaves in 10 ppm and 50 ppm SN solutions formed one major group (A) which indicates that leaves preserved in above two solutions showed almost similar trend to that of fresh leaves. Leaves preserved in distilled water, 200 and 100 µM STS solutions were placed on the other end (group C) indicating their negative impact on overall parameters, while leaves preserved in 100 ppm SN and 50 µM STS solutions (group B) representing moderate effect. On the horizontal axis three major groups (I, II and III) and one out group as mortality rate was formed. In one group (III) principal primary metabolites grouped with ERR (%) of rearing, in group II all the antioxidants were placed, while in group III rearing parameters along with secondary metabolites were grouped together. By analyzing all the groups formed it became clear that leaves that can retain the primary metabolites during preservation promote larval growth leading to better cocoon yield. Leaves where oxidative stress increases during preservation when allowed to feed upon larvae, decrease in growth index and economic attributes of cocoon takes place.

CONCLUSION

From our study it may be concluded that among the two silver salts (SN and STS), leaves that were preserved in SN solution more specifically in 10 ppm concentration were able to retain their physical and chemical characters as that of fresh leaves, indicating that as an anti-senescence agent, silver nitrate acts as a potent preservative for mulberry leaves. Besides this it was also found that silver nitrate preserved leaves when fed upon larvae, growth and cocoon parameters remained almost similar to that of fresh leaves. These findings might assist rural farmers to crop even during rainy season and might also motivate more people towards this field as they could carry out rearing for almost a week by collecting and preserving leaves once in a week.

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