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RESEARCH ARTICLE

TREATING THE LEAVES OF MULBERRY, *MORUS ALBA* (L) (V-1: VARIETY) WITH AQUEOUS SOLUTION OF MAPLE SYRUP BELONG TO *ACER SACCHARUM* (L) AND FEEDING THE FIFTH INSTAR LARVAE OF SILKWORM, *BOMBYX MORI* (L) (RACE: BIVOLTINE DOUBLE HYBRID (PO3 × ND5) × (CSR4 × CSR2) FOR THE QUALITATIVE SILK IMPROVEMENT

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ABSTRACT

The sugar maple tree is well known for its bright fall foliage and for being the primary source of sweet maple syrup. The maple syrup obtained from the sap of the sugar maple, *Acer saccharum*, is a popular sweetener consumed worldwide. Ten percent aqueous solution of maple syrup was utilized for treating the mulberry leaves. The maple syrup treated mulberry leaves were fed to the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: Bivoltine Double Hybrid (PO3 × ND5) × (CSR4 × CSR2). Treating the mulberry leaves with maple syrup and feeding them to fifth instar larvae was found reflected into significant improvement in the quality of the cocoons with 49 – 112 percent increase in the cocoon weight; 68 – 161 percent increase in the shell weight of the cocoon. The shell ratio of the cocoon spinned by the larvae fed with maple syrup treated mulberry leaves, were measured 22 – 24. The silk filament reeled from treated group was found significant with 38 – 80 percent increase in the length and 61 – 167 percent increase in the weight. The length and weight of silk filament reflect on the denier scale. The denier scale of silk filament of the treated group was found improved from 2.5 to 3.239. The contents of maple syrup belong to *Acer saccharum* (L) may serve to improve the digestibility and exert the influence on efficient metabolism in the fifth instar larvae of silkworm, *Bombyx mori* (L).The maple syrup treatment may gear overall biochemical constituency of silkworm larvae for the fortification of silk filament. The fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: Bivoltine Double Hybrid (PO3 × ND5) × (CSR4 × CSR2) should be fed with leaves of mulberry, *Morus alba* (L) supplemented with the sugar as in the present attempt. Use of herbal source for treating mulberry leaves and feeding the fifth instar larvae of silk worm, *Bombyx mori* (L) may be introduced in the rearing schedule. This may fortify the digestibility larvae and reflect into the qualitative silk production.

INTRODUCTION

The textile based and agriculture oriented sericulture industry largely characterized by wide variability's in cocoon yield performances. The variations in the cocoon yield performances are attributed to regional differences with climatic variations inherent both between and within the regions. The regional differences with climatic variations in turn have influence on mulberry leaf quality. The performance of silkworm larvae for the yield of cocoon crop is directly depend on the mulberry leaf quality. This is because mulberry leaves are the sole food for the growth and development of cocoon crops and better commercial characters on cocoons. The silkworm use to derive about seventy percent of the silk protein through the mulberry leaves and rest of the thirty percent of the silk protein from the silkworm body. Isn't it means "to focus on the nutritional qualities of the mulberry leaves" ? Silkworm is well recognized for its textile background and biological significance. It is economical and helpful insect and is reared by many farmers throughout world for commercial silk. The "Breed, Seed and Feed" seems to be the routes of the success in sericulture. The race or the breed of silkworm play crucial role for high cocoon yield and silk quality. The bivoltine race of breed of silkworm is superior with reference to high content of silk fibrion. Introduction of tropical bivoltine rearing practices in India is revolutionizing the bivoltine silk production in South India (Muniswamy, *et al* , 2016). The life of insect herbivores is in the orchestrate progression, which closely interlinked with plant metabolites. The biochemical constituents of plants could have been the factors of growth and metamorphosis for insects (Bowers *et al.*, 1966). The phytophagous insects are able to avoid poor quality food or able to select a high quanlitative food from variety available to

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them. The silkworm, *Bombyx mori* (L) is a monophagous insect, feeding exclusively on the leaves of mulberry *Morus alba* (L). It is therefore, essential to improve either food quality or appetite (or both) of larval instars of silkworm for better performance in silk production. The factors responsible to influence the growth, development and subsequent physiology of insect body include: nutritional qualities of food, biochemical status of nutrients in the food, hormonal level in the body and environmental conditions (Murugan and Georgr, 1992). Elements of the insect body are primarily derived from the food source. For silkworm, the leaves of mulberry contain many stimulants (Ito, 1960,1961; NayarandFraenkel, 1962; Ito, *et al*, 1964; Ito and Hyashiya, 1965). Nutrition quality in silkworm, *Bombyx mori* (L) serve to accelerate the growth, metamorphosis and forms the physiological foundation for sericulture. The leaves of mulberry are the sole source of food for larval instars of silkworm, *Bombyx mori* (L), biochemically constituted with proteins, lipids, carbohydrates (Murali, 1992) and minerals (Subramanyam Reddy, 1992). Therefore, corresponding diversity of enzymes capable of hydrolyzing the biocompounds of mulberry is exhibited by mid gut of larval instars of silkworm, *Bombyx mori* (L).

The body tissues of larval instars of silkworm, *Bombyx mori* (L) especially, the fat bodies accumulates large quantity of proteins, lipids and glycogen during the development, which is nothing but the reflection of efficient consumption and utilization of nutrient biocompounds of mulberry leaves. The variation in the food consumption in phytophagous insects may be for varied biochemical processes, ultimately for successful adaptations (Slansky, 1982). It has been suggested that, there is a functional difference between the activity of digestion by the digestive fluid in mid gut and tissue of mid gut. It has been reported by Horie, *et al* (1963) that, molecular proteins are hydrolyzed into peptides by digestive fluid content and into amino acids with peptidases in the mid gut tissue. Likewise, the polysaccharides, are digested in the insect gut lumen by digestive fluid and disaccharides and/or trisaccharides get hydrolysed into their constituent monasaccharide sugars mainly in the gut tissue (Horie, 1967).

The lipase, the lipid digesting enzyme of the insect mid gut has been reported to have analogy with pancreatic lipase of vertebrates (Yamafugi andYonezawa, 1935). The efforts towards the qualitative silk production through the improvement in the efficiency of consumption and utilization of food by larval instars of silkworm, *Bombyx mori* (L) include: improvement in the quality of mulberry leaves and supplementation of nutrient biocompounds like soya protein; potassium iodide, copper sulphate, other mineral salts, herbal products (or drugs) like digoxin (Vitthalrao and Kulkarni, 2011) andkho-go (Desai, *et al*, 2011). The polyphenols which also known as polyhydroxyphenols are the structural class of mainly natural chemical compounds. They are also in the form of synthetic or semisynthetic. They are the organic compounds characterized by the presence of large multiples of phenol structural units. The number and characteristics of these units of phenol in the structures underlie the unique physical, chemical, and biological (metabolic, toxic, therapeutic, etc.) properties of particular members of the class (Quideau, *et al* , 2011). The Canadian maple syrup is the source for isolation of "Quebecol" polyphenol (Li, Liya, *et al*, 2011). The "Quebecol" polyphenol is with the chemical formula $C_{24}H_{26}O_7$. It's systematic name 2,3,3-tri-(3-methoxy-4-hydroxyphenyl)-1-propanol. According to Johnson, Tim (2011), the "Quebecol" polyphenol compound is not naturally present in the sap. Instead, it is formed during extraction or processing. Cardinal, Sébastien and Voyer (2013) has reported the norm and total synthesis of the "Quebecol" polyphenol compound. Sébastien Cardinal *et al*. (2017) and Normand Voyer (2017) reported that, the isoquebecol (2,3,3-tri-(3-hydroxy-4-methoxyphenyl)-1-propanol) has an improved anti-inflammatory activity (ability to inhibit IL-6 secretion) over that of its natural isomer quebecol. The name "Quebecol" to this polyphenol compound is in honour of the Canadian province of Quebec which is the world's largest producer of maple syrup. The sugar maple tree is well known for its bright fall foliage and for being the primary source of sweet maple syrup. The sap of maple, *Acer saccharum* (L) is the sole source of the maple syrup (Brisson, *et al*, 1994). Near about 80% of the world's syrup comes from Quebec.

It was natural that when N. P. Seeram and co-workers at the University of Rhode Island (Kingston) isolated a previously unknown phenolic alcohol from the syrup, they named it quebecol. Seeram and co-workers believe that quebecol does not exist in the sap but is formed during syrup production. Several researchers at the spring 2014 ACS National Meeting, tried their best to discuss their work on the possible health effects of "Quebecol" belong to maple syrup. Recent studies have reported that, maple syrup contains many antioxidative phenolic compounds Li L, Seeram,2010;2010; and Zhang, *et al*, 2011). The digestibility in the fifth stage larval stages of *B. mori* correspond to food material ingested. The herbal sweetener, stevia inulin through treating mulberry leaves and feeding fifth instar larvae of was found yielding the qualitative cocoons and silk filament in the polyvoltine crossbreed (PM x CSR_2) (Shubhangi Pawar and Vitthalrao Khyade, 2017). For the purpose to analyze the effect of treating the leaves of mulberry, *Morus alba* (L) with aqueous solution of maple syrup and feeding the fifth instar larvae of silkworm, *Bombyx mori* (L)(Race: Bivoltine Double Hybrid (PO3 × ND5) × (CSR4 × CSR2) the present attempt has been planned.

MATERIAL AND METHODS

The attempt has been carried through the steps like: Rearing of the larval instars of silkworm, *Bombyx mori* (L); Preparation of solution of maple syrup; Treating the mulberry leaves with maple syrup solution; Feeding the larval instars of silkworm; Provision of mountages to the mature fifth instar larvae of silkworm for spinning the cocoon; Harvesting the cocoons; Analysis of the parameters of cocoon and silk filament and Statistical analysis of the data. The disease free layings (DFL) of silkworm, *Bombyx mori* (L) (Race: Bivoltine Double Hybrid [(PO3 × ND5) × (CSR4 × CSR2)] were procured through the sericulture unit of Agriculture Development Trust, Malegaon (Baramati). The disease free layings were processed for black boxing for incubation; transfer of hatched larvae on the rearing bed of mulberry leaves and reared through the methods prescribed by Krishnaswami, *et al* (1978) and explained by Khyade (2004) through the use of leaves of mulberry, *Morus alba* (L) (M-5: variety). Soon after the fourth moult, the fifth instar larvae were divided into various groups like untreated control (UTC), water treated control (WTC) and the three groups of larvae for feeding the maple syrup treated mulberry leaves [(MSTF-I); (MSTF-II) and (MSTF-III)]. Each group of the fifth instar larvae was with 100 individuals. As per the standard schedule, four feedings were followed (6 am, 11 am, 4 pm and 10 pm). For each feeding, for the group of hundred fifth instar larvae, 100 grams of fresh leaves of mulberry, *Morus alba* (L) (M-5: variety) were utilized. The bottle of maple syrup belong to *Acer saccharum* (L) was procured from local medical store and utilized for treating the leaves of mulberry, *Morus alba* (L) (Variety: V-1) collected from Malegaon Sheti farm of Agriculture Development Trust. forty milliliters (40 ml) of maple syrup mixed in 360 milliliters known volume of distilled water to prepare aqueous solution of ten percent strength. The stock solutions of syrup were prepared freshly before the feeding. 400 ml of aqueous solution of maple syrup formulation was used to treat 100 grams of fresh mulberry leaves. The mulberry leaves were immersed in the maple syrup solution. mulberry. The treated group of fifth instar larvae , entitled, MSTF-I were fed with maple syrup treated mulberry leaves for the first day only. The treated group of fifth instar larvae , entitled, MSTF-II were fed with maple syrup treated mulberry leaves for the first and the second day only. The treated group of fifth instar larvae , entitled, MSTF-III were fed with maple syrup treated mulberry leaves for the first day; the second day and the third day (Table-1). For remaining days, the larvae were fed with untreated leaves of mulberry. The matured fifth instar larvae (with transparent skin, non-feeding and moving their head in specific manner for searching the surface for attachment of fluid silk) were transferred to the plastic mountage for spinning the cocoon. The cocoons were harvested on fifth day after mounting the mature larvae on the plastic mountage.

Table 1. Schedule of Feeding the leaves of mulberry, Morus alba (L) (V-1 Variety) treated with aqueous solution of maple syrup to the fifth instar larvae of silkworm, Bombyx mori (L) (Race: Bivoltine Double Hybrid (PO3 × ND5) × (CSR4 × CSR2)

Day of Feeding→ Group↓	First	Second	Third
Untreated Control (UTC)	-	-	-
Untreated Control (WTC)	-	-	-
(MSTF-I)	+	-	-
(MSTF-II)	+	+	-
(MSTF-III)	+	+	+

UTC=Untreated Control; WTC =Water Treated; MSTF=Mapple Syrup Treated Leave Feeding; -: Feeding with Untreated Leaves; + : Feeding with Mapple Syrup Treated Leaves; - : Feeding with Water treated Leaves.

Table 2. The economic parameters of the cocoons and silk filament spinned by mature fifth instar larvae of silkworm, Bombyx mori (L) (Race: Bivoltine Double Hybrid (PO3 × ND5) × (CSR4 × CSR2) fed with leaves of mulberry, Morus alba (L) (V-1 Variety) treated with aqueous solution of maple syrup

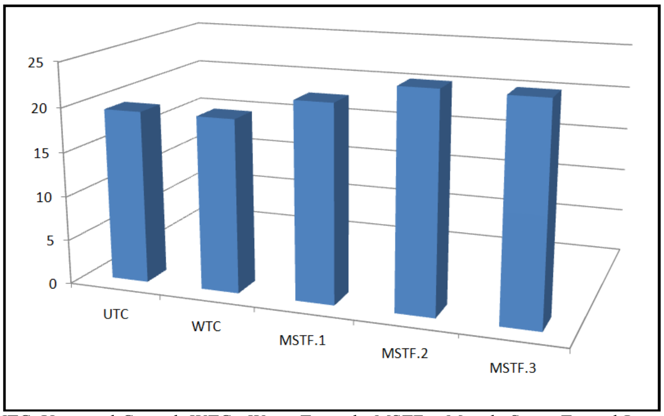
Parameters→ Group↓	Cocoon Weight (gm)	Shell Weight (gm)	Pupal Weight (gm)	Shell Ratio	S F L (m) (A)	S F W (gm) (B)	Denier Scale of S F = (B÷A) x 9000
UTC	1.869 (±0.088) 00.000	00.364 (±0.007) 00.000	1.505 0.000	19.475 0.000	813.55 (±11.337) 00.000	0.197 (±0.038) 00.000	2.179 00.00
WTC	1.869 (±0.093) 00.000	00.364 (±0.018) 00.000	1.505 0.000	19.475 0.000	813.55 (±14.561) 00.000	0.197 (±0.041) 00.000	2.179 0.000
MSTF.1	2.786* (±0.079) 49.063	00.613** (±0.031) 68.406	2.173**	22.002*	1128.38** (±23.196) 38.698	0.319** (±0.048) 61.928	2.544**
MSTF.2	3.234* (±0.119) 73.033	00.782* (±0.111) 114.83	2.452**	24.180**	1439.98** (±127.33) 76.999	0.508** (±0.084) 157.86	3.175**
MSTF.3	3.963*** (±0.786) 112.038	00.952*** (±0.138) 161.53	3.011***	24.022***	1464.23*** (±129.47) 79.980	0.527*** (±0.088) 167.51	3.239***

-Each figure is the mean of the three replications.
-Figure with ± sign in the bracket is standard deviation.
-Figure below the standard deviation is the increase for calculated parameter and percent increase for the others over the control.
UTC=Untreated Control; WTC =Water Treated Control; SFL= Silk Filament Length; SFW= Silk Filament Weight ; MSTF = Mapple Syrup Treated Feeding
* : $P < 0.05$;** : $P < 0.005$; ***: $P < 0.01$

The treating the mulberry leaves was carried out for half an hour before feeding. The soaked/ treated mulberry leaves were drained off completely and then fed to the fifth instar larvae of silkworm, *Bombyx mori* (L). Hundred grams of mulberry leaves were utilized for feeding each time, for the group of hundred larvae. The untreated control group of larvae were supplied with untreated leaves of mulberry. Water treated group of larvae were supplied with water treated leaves of Cocoon weight, shell weight and pupal weight were recorded. Shell ratio was calculated. Ten cocoons per replication were reeled by using the eprouvate or wheel charakha for silk filament. The length (m) of unbroken silk filament from individual cocoon was recorded. Weight of silk filament from individual cocoon was recorded. The readings on length (m) and weight (gm) of silk filament were accounted for the calculation of Denier scale. The experimentation was repeated for thrice for the purpose of consistency in the results. The statistical methods were employed to calculate the mean, standard deviation, percent variation and student "t" – test (Norman and Bailey, 1955). The data collected belongs to three successive trials.

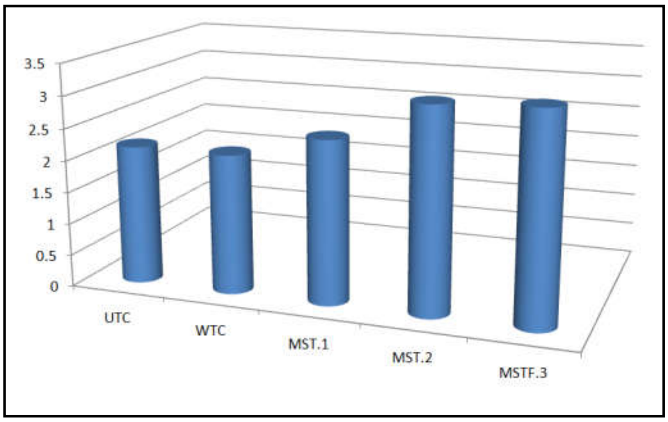
RESULTS AND DISCUSSION

The results on the treating the leaves of mulberry, *Morus alba* (L) (M-5: variety) with aqueous solution of maple syrup and feeding the fifth instar larvae of silkworm, *Bombyx mori* (L) (Race: Bivoltine Double Hybrid (PO3 × ND5) × (CSR4 × CSR2) for the qualitative silk yield are summerised in table 2 ; presented in Fig. 1 and Fig.2.



UTC=Untreated Control; WTC =Water Treated ; MSTF = Mapple Syrup Treated Leave Feeding;
- : Feeding with Untreated Leaves;
+: Feeding with Mapple Syrup Treated Leaves;
- : Feeding with Water treated Leaves.

Fig. 1. The shell ratio of the cocoon spinned by mature fifth instar larvae of silkworm, *Bombyx mori* (L) (Race : BIVOLTINE DOUBLE HYBRID [(PO3 × ND5) × (CSR4 × CSR2)]) fed with leaves of mulberry, *Morus alba* (L) treated with the aqueous solution of maple syrup



UTC=Untreated Control; WTC =Water Treated ; MSTF = Mapple Syrup Treated Leave Feeding;
- : Feeding with Untreated Leaves;
+: Feeding with Mapple Syrup Treated Leaves;
-  : Feeding with Water treated Leaves.

Fig. 2. The Denier scale of silk filament reeled from the cocoons spinned by mature fifth instar larvae of silkworm, *Bombyx mori* (L) (Race : BIVOLTINE DOUBLE HYBRID [(PO3 × ND5) × (CSR4 × CSR2)]) fed with leaves of mulberry, *Morus alba* (L) treated with the aqueous solution of maple syrup

Treating the mulberry leaves with ten percent aqueous solution of maple syrus belong to *Acer saccharum* (L) and feeding them to the fifth instar larvae of silkworm, *Bombyx mori* (L) for first day (Group: MSTF-I); for the first day and the second day (Group: MSTF-II); and for the first day and the second day three days; second and third days (Group: MSTF-III) was found variously reflected into the changes in the weight of cocoon, shell weight, shell ratio, silk filament length, silk filament weight and denier scale of the silk filament. The weight (grams) of entire cocoon, shell weight (grams), shell ratio (Percentage) and pupal weight(grams) of the Untreated Control group (UTC) were found measured 1.869 (±0.088); 0.364 (±0.088); 19.475 and 1.505 respectively. The weight (grams) of entire cocoon, shell weight (grams), shell ratio (Percentage) and pupal weight(grams) of the Water Treated Control group (WTC) were found measured 1.869 (±0.093); 0.364 (±0.018); 19.475 and 1.505 respectively. The weight (grams) of entire cocoon, shell weight (grams) , shell ratio (Percentage) and pupal weight(grams) of the Group: MSTF-I (Fed with maple syrup treated mulberry leaves for the first day of fifth instar larvae of silkworm, *Bombyx mori* L) were found measured 2.786 (±0.079); 0.613 (±0.031); 22.002 and 2.173 respectively. The weight (grams) of entire cocoon, shell weight (grams) , shell ratio (Percentage) and pupal weight(grams) of the Group: MSTF-II (Fed with maple syrup treated mulberry leaves for the first day and the second day of fifth instar larvae of silkworm, *Bombyx mori* L) were found measured 3.234 (±0.119); 0.782 (±0.111); 24.180 and 2.452 respectively.

The weight (grams) of entire cocoon, shell weight (grams) , shell ratio (Percentage) and pupal weight(grams) of the Group: MSTF-III (Fed with maple syrup treated mulberry leaves for the first day, the second day and the third day of fifth instar larvae of silkworm, *Bombyx mori* L) were found measured 3.963 (±0.786); 0.952 (±0.138); 24.022 and 3.011 respectively. Treating the leaves of mulberry with aqueous solution of maple syrup belong to *Acer saccharum* (L) and feeding them to the fifth instared larvae of silkworm, *Bombyx mori* (L) (Race: Bivoltine Double Hybrid (PO3 × ND5) × (CSR4 × CSR2) was found significantly improving the weight of entire cocoon from 49 to 112 percentage (Table – 1 and Fig. – 1). Silk is a natural protein fiber, some forms of which can be woven into textiles. It is the sole aim in sericulture. The length (Meters); weight (Grams) and the Denier scale of the silk filament reeled from the cocoons of untreated control (UTC) in the attempt were found measured 813.55 (±11.337); 0.197 (±0.038) and 2.179 respectively. The length (Meters); weight (Grams) and the Denier scale of the silk filament reeled from the cocoons of water treated control (WTC) in the attempt were found measured 813.55 (±14.561); 0.197 (±0.041) and 2.179 respectively. Treating the leaves of mulberry, *Morus alba* (L) with the maple syrup and feeding them to the fifth instar larvae of silkworm, *Bombyx mori* (L) for the first day (Group: MSTF-I) was resulted into significant improvement in the silk filament with length measuring 1128.38** (±23.196) units; weight measuring 0.319** units and denier scale with 2.544** . The silk filament reeled from the cocoons harvested from the group of larvae fed with treated mulberry leaves for two days (Group: MSTF-II) was with length measuring 1439.98** (±127.33) units ; weight of 0.508** (±0.084) and with denier scale of 3.175**. The most significant results on the silk filament parameters belong to the group MSTF-III (fed with maple syrup treated mulberry leaves for the first day; the second day and the third day). The length (M), weight (grams) and the denier scale of silk filament reeled from the cocoons belong to the larvae fed with maple syrup treated mulberry leaves for the first day; the second day and the third day, were measuring 1464.23*** (±129.47); 0.527*** (±0.088) and 3.239***. In the mulberry, *Morus alba* (L), it is well established fact regarding, "Younger the leaf higher the proteins and lower carbohydrate contents" (Cui, *et al*, 2008). The early instar (the first, second and third) larvae of silkworm, *Bombyx mori* (L) are fed with younger mulberry leaves to fulfill the protein requirements. The late instar (the fourth and fifth) larve are fed with the tender mulberry leaves (Khyade, 2004). The sugar content of tender leaves of the mulberry, *Morus alba* (L) is more in comparison with the proteins. The tender leaves of mulberry, *Morus alba* (L) with higher content of carbohydrates are serving a lot to orchestrate the metabolic turnover for the amino acids for synthesis of fibrion, the silk protein. The mulberry leaves should be fortified with carbohydrate suppliments as in the present attempt for enhancing the growth and maturity of larval instars of silkworm, *Bombyx mori* (L).

The final larval instars of lepidopteran insects like silkworm, *Bombyx mori* (L) have four phases of growth which include: preparatory (first two days); Accumulation phase (third and fifth days); Regression phase (sixth day) and Degeneration phase (day of spinning). The initial preparatory phase is characterized by high rate of DNA synthesis, high rate of digestion, moderate RNA synthesis and low protein synthesis. This phase seems to be juvenile hormone dependent. Accumulation phase, regression phase and degeneration phase are concerned mainly with silk glands. Improvement in the quality of the cocoons and the silk filament reeled from them, in the group of larvae fed with maple syrup treated mulberry leaves, in the present attempt with bivoltine Double Hybrid [(PO3 × ND5) × (CSR4 × CSR2)] seems to be affecting the growth phases of larva. Treating the mulberry leaves with herbal drug: maple syrup belong to *Acer saccharum* (L) and feeding them to the fifth instar larvae of silkworm, *Bombyx mori* (L) for continuous three days (First, Second and Third) appears to be significant in comparison with others. Feeding the larvae continuously for the three days with maple treated mulberry mulberry leaves may be positively affecting on digestibility of larvae. This may be contributing for extra synthesis of the silk and making the larvae efficient for spinning qualitative cocoons with fortified silk filament.

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