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Use of leaves of mulberry, *Morus alba* (L) treated with Stevia Inulin for the improvement of activities of enzymes in the mid gut protease and amylase of the last stage silkworm larvae

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Abstract

The inulin is a heterogeneous collection of fructose polymers anda soluble dietary fiber. The present attempt is concerned with use of leaves of mulberry, Morus alba (L) treated with Stevia Inulin for the improvement of activities of mid gut protease and amylase in last stage silkworm larvae. Four different concentrations of aqueous solution of herbal formulation: Stevia inulin powder (5.0 mg/ lit; 10.0 mg/ lit; 20.0 mg/ lit and 50.0 mg/ lit) were used to soak the leaves of mulberry. The treated leaves were used feeding. The schedule of feeding for each strength of solution for the first group (A-First; B-First; C-First and D-First) was for the three days (First; Second and Third); For second group (A-Second; B-Second; C-Second and D-Second)) was for two days (second and third) and for the third group (A-Third; B-Third; C-Third and D-Third) was only for one day (third). The analysis of biochemical parameters was carried through the use of fifth stage instars of five days old. The biochemical characters considered for analysis were proteins (Soluble fraction of Proteins and Total fraction of Proteins). The activities of enzymes, namely protein digesting enzyme (protease) and carbohydrate digesting enzyme (amylase) in were also considered in the present attempt. The tissue used for the biochemical analysis was mid gut homogenate. Soaking the mulberry leaves with Stevia inulin powder and supplying the treated leaves to last larval instar was found effected into significant changes contents of both soluble and total proteins. The activities of protein digesting enzyme (protease) and carbohydrate digesting enzyme (amylase) were also found effected through the inulin treatment. The contents of soluble proteins and total proteins of mid gut homogenate pattern was found increased respectively from 32.147 percent to 90.074 percent and 5.657 percent to 39.052 percent. The activities of protein digesting enzyme (protease) and carbohydrate digesting enzyme (amylase) were observed to increase from 21.444 to 83.706 and from 14.540 to 54.257 percent respectively. The composition of Stevia inulin powder serve to fortify the digestion. It may also exert the effects on activity in metabolism in the last stage larval of silkworm, Bombyx mori (L). Inclusion of the method of "Treating the leaves of mulberry with Stevia inulin powder before the feeding" may be effecting to speed up the rate of metabolism in the last larval instars of silkworm. Utilization of Stevia inulin powder for soaking the leaves of mulberry before supplying to the last larval stage of silkworm, Bombyx mori (L) may be considered for introduction in the "Methodlogy of Sericulture. It may help to improve the health of silkworm.

Keywords: Silkworm, *Bombyx mori*, Stevia, Inulin, Midgut enzymes.

Introduction

The plant metabolites are serving for progressive orchestration of the life in the animals like the insects. The life of insects feeding on the herbal material is closely associated with the metabolic contents of the plants. According to Bowers, *et al*[1], the factors governing the growth and metamorphosis in the insects could be the chemical contents of herbal material used by the for eating. Insects are supposed lucky with reference to availability of rich source of herbal food material. This is because, the insects are capable to select the qualitative food which is available for them in the form of rich herbal flora. They are also able to avoid the herbal material with poor quality. Special feature of insects lies in having the significant stages in the life cycle. The stages in insect life cycle include: Egg stage; the stages of larvae; cocoon stage and the adult moth. Inside the cocoon, there is pupa. The stage of pupa may be with cocoon shell or it may be naked. The life of silkworm larvae is depending only on the leaves of mulberry, *M. alba* for the nutrient food material. This is the reason to label the silkworm, *B. mori* as "Monophagous". Sericulture, or silk farming, is the cultivation host plants and rearing larval stages of silkworms for commercial silk. Qualitative attempts are therefore, needed for the improvement of host plant; health of larval stages of silkworm. Healthier host plant mulberry, *Morus alba* (L) is known to yield qualitative leaves to be used for feeding the early and late instars of *B. mori*. And healthier silkworm larvae exert better performance in synthesizing the silk and spinning the silk. There are many other factors that govern the larval performance for yielding the silk. The quality of nutrition;

physiological status of the nutrients in the food material; levels of hormones in the body of larval stages of silkworm and the available conditions of environment constitute the "Governing set of factors" for the life of herbivorous insects like, silkworm, *Bombyx mori* (L)[2]. The herbal food material is alone source of each and every body element in the tissues in the body of herbivorous insects. The leaves of mulberry, *Morus alba* (L) deserve many stimulant chemicals that are essential for the life of silkworm, *Bombyx mori* (L)[3-6]. Better quality of mulberry leaves is prime concern for the life of silkworm, *Bombyx mori* (L). This is because, quality of the nutrients in the larval body exert accelerated status of growth and larval metamorphosis in silkworm, *Bombyx mori* (L). Moreover, quality of mulberry leaves gets reflected into the quality larval health in silkworm, *Bombyx mori* (L). This principle may be used to fortify the basics foundations of physiology of larvae of *B. mori* (L). Leaves of mulberry are constituting the alone store house of nutrients accounted in the life of larval stages of the silkworm-insect, *B. mori*. According to Murali[7], the leaves of mulberry, *Morus alba* (L) contain protein; fat and sugars. Mineral contents of mulberry, *Morus alba* (L) is also well established biological fact[8]. Rich contents of nutrients in the mulberry leaves made the larval stages of silkworm, *Bombyx mori* (L) to establish diverse range of enzymes that are able to digest and utilize each and every nutrient component. The credit of accumulation of significant amount of nutrients in the form of "Proteins; Lipids and Carbohydrates" in the silkworm larvae goes to the fat bodies. The carbohydrates in the fat bodies in silkworm larvae are in the form of glycogen. One more feature of the insect fat bodies is the concentration of the nutrient reserves correspond to the stage of in the metamorphosis. That is to say about the significant efficiency of regarding consumption and utilization of mulberry constituents by the silkworm larvae. Variations for the consumption of ingestion of food material in leaf eating insects is to deserve diversified processes of metabolism[9]. Retention of diversified metabolism by the life stages of insect is for the purpose of successful adaptations. There is variation in the process of digestion through differentiated the digestive fluid of insect mid gut and tissue of insect mid gut[10]. Further, Horie, *et al*[10] reporting the peptidases that concerned with hydrolysis of molecular proteins into peptides through the action of mid gut digestive fluid into simple amino acids. Likewise, the carbohydrate polysaccharides, liable for digestion in the lumen of insect mid gut lumen through the digestive fluid. Same is the case for and disaccharides and/or even, trisaccharides. The enzyme – Lipase is concerned with the lipid digestion. The special feature of enzyme lipase from insect mid gut fluid lies in it's analogy with lipase in the vertebrate pancreatic juice[11]. Some of earlier attempts for the silk yield qualitatively and quantitatively include upgradation of abilities of ingestion of food material and it's efficient use for the contribution to fortify the tissues of the body of silkworm larvae; fortification of the quality of mulberry leaves of mulberry; supplying the of the nutrients through the food material; Treatment of silkworm feed with proteins like soya proteins and feeding such soya treated mulberry leaves to silkworm larvae; Iodizing the mulberry leaves through potassium iodide (KI); Treating mulberry leaves with chemicals like copper sulphate, other mineral salts, plant derived nutrients and or medicines drugs like lanoxin[12] and cough syrup[13]. And then use of such treated mulberry leaves to the larval stages of silkworm, *Bombyx mori* (L).

The sweet leaf, *Stevia breboudiana* Bertoni is known for containing Steviol glycosides. The steviol deserve sweet taste. One more feature of steviol is it's stableness for the heat and pH. Steviol do not ferement and structurally, it is a diterpene, aglycone unit of the sweet glycoside (Stevoside) of *Stevia reboudiana* Bertoni[14,15]. The credit of extraction and evaluation (as a sweetener) of steviol glycoside through the use of leaves of *Stevia breboudiana* Bertoni goes to the European Food Safety Authority. This European Food Safety Authority tried it's best to express the units of steviol for human consumption for the betterment of health (Commission Regulation / EU / No. 1131 / 2011). Medicinally important status of Steviol; it's glycoside nature (glycoside treated mulberry leaves may accelerate the rate of midgut enzyme catalyzed biochemical reactions in the larval instars of silkworm). Most of the Juvenoids utilized to treat the larval instars of silkworm through topical application are terpenoids. Glycoside nature of steviol; it's diterpene structure and it's pharmaceutical status made to plan for the present attempt of the study.

Materials and methods

The attempt of present study was carried out through the parts that include: Preparation of the steviol solution; Rearing of silkworm larvae; treating the leaves of mulberry with steviol solution. Use of such steviol treated mulberry leaves for feeding silkworm larvae; Biochemical analysis and analysis of the data through statistical methods.

Preparation of the steviol solution: The commercial Stevia inulin powder was purchased through local supplier of medicines. It was processed for the preparation of aqueous solution. Known volume of distilled water as a solvent was taken. Appropriate amount of stevia inulin powder was dissolved in solvent. Different concentrations of stevia inulin in water considered in the present attempt include: 5 mg/Lit. (5 part per million); 10 mg/Lit. (10 part per million); 20 mg/Lit. (20 part per million) and 50 mg/Lit. (50 part per million). Fresh aqueous solutions of stevia inulin powder preparation was used in the study.

Rearing of silkworm larvae: Egg cards, entitled "Disease Free Layings (DFL)" of silkworm was brought from the sericulture study centre at "Agriculture Development Trust, Malegaon (Baramati)". The race of silkworm used in the study was the polyvoltine, crossbreed race: PM x CSR_2. The egg cards (DFLs) were used for processing incubation. This incubation was carried out by keeping the egg card in the black box (black boxing). The larvae emerged from eggs were transferred to the plastic tray with leaves of mulberry, *Morus alba* (L). The larvae

were fed four time for each day with appropriate amount quality and quantity. At the time of moulting, care of the larvae was taken through the use of lime powder. The standard methods of silkworm rearing were followed in present attempt of study[16].

Stevia Inulin treatment of leaves of mulberry: The larvae soon after the fourth moulting were considered as of fifth stage instars. The larvae were divided for the purpose to make different groups (Table-1). Each group was consisted of hundred individuals. The groups of larvae include untreated control (UTC); water treated control (WTC) (O-I; O-II; O-III) and the treated groups (A-I, A-II, A-III; B-I, B-II, B-III; C-I, C-II, C-III and D-I. D-II, D-III) (12). The larvae in each group were fed with mulberry leaves for four times each day. The feeding times include: 6 am, 11 am, 4 pm and 10 pm. For the group of hundred larvae, for each feeding, hundred grams of leaves (fresh) of mulberry leaves were used. The water solutions of stevia inulin of each concentration (5 ppm; 10 ppm; 20 ppm and 50 ppm) were prepared freshly. The volume of aqueous stevia solution utilized for the treatment of hundred grams of mulberry leaves was four hundred milliliter. Hundred grams fresh mulberry leaves were kept immersed in hundred milliliter stevia inulin solution of known strength for respective group. The leaf treatment was for half an hour before feeding. After the schedule of treatment, the leaves of mulberry were allowed for draining off completely. After draining completely, the treated leaves of mulberry were used for feeding the larval groups in the study. The group of hundred larvae was supplied with hundred grams of mulberry leaves for feeding for each time. Larvae belonging to "Untreated control (UTC) group" was maintained through the provision of mulberry leave without any treatment. Larvae belonging to "Water treated control (WTC) group" were provided with mulberry leaves treated with distilled water. For each strength (mg/Lit. or ppm) of stevia inulin, three different larval groups (A-I, A-II, A-III for 5 ppm; B-I, B-II, B-III for 10 ppm; C-I, C-II, C-III for 20 ppm and D-I, D-II, D-III for 50 ppm stevia solution) were made. The larval groups A-I, B-I, C-I and D- I were fed with mulberry leaves treated with stevia inulin solution for the first, second and third days. The larval groups A-II, B-II, C-II and D- II were fed with mulberry leaves treated with stevia inulin solution for the second and third days. The larval groups A-III, B-III, C-III and D- III were fed with mulberry leaves treated with stevia inulin solution for only third day (Table-1). For remaining days, the larvae were fed with untreated leaves of mulberry.

Biochemical analysis: Fifth day of the fifth instar larvae of silkworm was preferred for biochemical analysis[17]. The mid gut homogenate was utilized for biochemical analysis. From each group in the study, twenty larvae were selected randomly. Through the use of chloroform soaked cotton pads, individual larva was anaesthetized. Individual larva was used for dissection in 0.9 percent sodium chloride solution. The gut of individual larva was separated. The midgut from entire gut was separated. It was the flushed through the use of ice cold saline that is 0.9 percent sodium chloride solution. The ice cold saline flushing of the midgut is for separation of mulberry leaf debris. Separate individual midgut was used for fragmentation followed by homogenization. It was followed by centrifugation. The temperature and the rate of centrifugation was 40^0C for 15 min. at 10000 rpm respectively. The supernatant obtained was considered for equalization to the required volume. Aliquots of supernant was of the strength of 10 mg per ml. This volume was used as assay sample. Half of volume of assay sample was used for bioassay of soluble proteins. Remaining half of the volume was considered assaying mid gut enzymes. The midgut enzymes considered for bioassay in the present attempt were protease and amylase.

Table-1: Schedule of treating the mulberry leaves with aqueous solution of Stevia inulin powder herbal formulationand feeding to the fifth instar larvae of silkworm, *Bombyxmori* (L) (Race: PM x CSR_2).

Group	Day for feeding→ Concentration of stevia inulin(ppm)↓	1	2	3
0-0	Untreated control	-	-	-
0-I	Water treated control	□	□	□
0-II	Water treated control	-	□	□
0-III	Water treated control	-	-	□
A-1	5ppm	+	+	+
A-2	5ppm	-	+	+
A-3	5ppm	-	-	+
B-1	10ppm	+	+	+
B-2	10ppm	-	+	+
B-3	10ppm	-	-	+
C-1	20ppm	+	+	+
C-2	20ppm	-	+	+
C-3	20ppm	-	-	+
D-1	50ppm	+	+	+
D-2	50ppm	-	+	+
D-3	50ppm	-	-	+

– = Untreated mulberry leaves. □ = Water treated mulberry leaves, + = Steviol inulin Powder treated mulberry leaves.

The bioassay of soluble proteins and total proteins was carried through the use of standard method of Lowery *et al*[18]. For each sample from each group in the study, triplicate set was prepared. The sequence of additions in each test tube was: Assay Sample (one ml) and Lowery's "C" solution (five milliliters). Simultaneously, a set of "the blank test tubes" was also prepared. In test tubes of "the blank set", distilled water was considered instead of the assay sample. The test tubes were

shaked for mixing the contents. This preparation was kept for about fifteen minutes. This was for the purpose of formation of copper-protein complex. Thereafter, additions of solution pertaining reagent of Folin-Phenol (0.5 ml) in each test tube was carried out. All the test tubes were shaked well. The content thus obtained was kept for color development. Spectrophotometer was used for the purpose to read the optical density (OD). 660 nm wavelength was the utilized for optical density readings. Standard graph pertaining the concentration of BSA against the optical densities readings was referred for the purpose to know the concentration of protein in each of sample of assaying. "Microgram protein per mg tissue" was the unit to express the contents of proteins in each of sample of assaying.

Another set of twenty five larvae form each group was considered for the bioassay of the total proteins. The larvae were selected randomly. With the help of chloroform soaked cotton pads, the larvae were anaesthetized. The larvae were dissected to separate the mid gut tissue. Individual mid gut tissue was homogenated through the use of morter and pestle. Before the use, morter and pestle were kept in chilled distilled water followed by keeping in one normal (1.0 N) solution of sodium hydroxide. This was for the purpose to sterilize. Then, the morter and pestle were kept in freeze at 37^0C for 24 hours. Then the set was utilized for homogenizing the tissue of mid-gut. The homogenized content was the processed for precipitation with same volume of ten percent solution of TCA. This content was then processed for centrifuged at 10000 rpm for 10 minutes. 1.0 NaOH solution was used for dissolution of the precipitate. The content thus obtained was considered as assay sample for total proteins. Further steps in the total proteins assessment of assay sample are the same as that explained earlier for soluble fraction of proteins.

The analysis of protease enzyme activity in mid-gut tissue was carried out through the use of standard method of Brik, et al[19]. Isshaya *et al*[20] suggested slight modification in the Brik, et al[19]. This slight modification in the midgut protease was outlined by Alka Chougale[21]. This modification was considered in the present attempt. Three test tubes were considered for each group in the attempt, that is to say triplicate set of bioassay. Blank set was also prepared along with the attempt. The contents for incubation in test tube consisted of one ml of ten percent casein solution (as a substrate); 0.5 ml assay sample (as a source of enzyme) and 0.5 ml of 0.2M Trisbuffer of pH= 8.4. Instead of assay sample, distilled water was used in the set of the blank. Individual set of test tubes were kept for the incubation. Water bath was used for incubation of bioassay mixture. This incubation was carried out at 30^0C for 20 minutes. The incubation set was allowed for constant shaking. After incubation, there was addition of 6 ml of 2 percent TCA in each test tube. It was followed by centrifugation of the content. Centrifugation was carried at 8000 rpm. Time for centrifugation was fifteen min. The supernatant thus obtained was used for reading the optical density (OD) at 280 nm on spectrophotometer. The standard graph on the concentration of tyrosine and the readings of optical density (OD) was used for the calculation of amount of tyrosine liberated in each assay sample. The tyrosine liberated from the casein through the activity of mid gut protease was calculated by using the readings of optical density (OD) for each assay sample. The predetermined soluble protein contents of each assay sample were also considered for knowing the activity of mid gut protease. Units of specific activity for protease enzyme expressed in the present study were: microgram tyrosine released per mg protein per minute.

The method of Bernfeld[22] with modifications suggested by Gaikwad[23] and outlined by Khyade[17] and Desai, *et al*, [13] was used for the determination of activity of amylase enzyme from the assay sample belong to the mid gut of fifth instar larvae of silkworm, *Bombyx mori* (L). For this purpose twenty larvae from each group were selected randomly. These larvae were processed for preparation of assay sample described earlier for soluble proteins. The bioassay was carried in triplicate set Mid (along with blank). The incubation mixture in the test tube was consisted of one ml of substrate (one percent starch solution) (as substrate), buffer solution (phosphate buffer with pH=9.2) and assay sample (0.5 ml) (as a source of enzyme). Distilled water was used instead of assay sample for the blank set of bioassay. The mixture was processed for incubation in water bath at 30^0C for 20 minutes. The enzyme activity was terminated through the addition of 2 ml of DNSA solution and 2 ml of distilled water. This mixture was heated in boiling water bath exactly for five minutes and cooled immediately. The final content was the used for reading the optical density (OD at 540 nm on spectrophotometer.

The optical density readings for each assay sample; standard solution of maltase (from graph) and soluble proteins were used for the purpose to know activity of amylase in the mid gut homogenate. Units of specific activity for amylase enzyme expressed in the present study was: micrograms of maltose liberated per mg protein per minute.

Analysis of the data through statistical methods: All the attempts in the study were subjected for repetition. The purpose of repetition was to obtain the consistency in the results of the experimentations. The data from each attempt was collected. Mathematical statistical methods of analysis were followed for the calculation of the mean; standard deviation; percent change and tests of significancy. The statistical method used in the study belongs to Norman and Bailey[24]. The student "t" – test was utilized for knowing the significant changes in the treated groups and comparison with the control group.

Results and discussion

The results on the influence of feeding stevia inulin treated mulberry leaves on the mid gut homogenate in the fifth stage larvae of multivoltine, crossbreed race of silkworm, *Bombyx mori* (L) are summarized through the Table-2. The Stevia inulin

treatment to the mulberry leaves and supplying them to the silkworm fifth larval instars, for first day second day and third day (Groups: A-I, B-I, C-I and D-I); second and third days (Groups: A-II, B-II, C-II and D-II); and for third day (only) (Groups: A-III, B-III, C-III and D-III) was found variously effected in changes in the quantity of both soluble and total proteins and the velocity of activities of protease enzyme and amylase enzyme in the mid gut tissue homogenate. The soluble proteins respectively for the group untreated control (0-0) and the group water treated control (0-I) were measured 138.830 (+ 49.850) and 135.110 (+62.485). The water treated control groups for second day and third day (0-II) and for only third day were with 137.17 (+54.668) and 137.69 (+53.579) units of soluble proteins in the homogenate of mid gut tissue. Total protein content of the mid gut tissue of larvae received untreated mulberry leaves (UTC group) were measured 578.43 (+127.51) units. The group of larvae received water treated mulberry leaves for first three days; second day and third day, only for third day were found 567.14 (+158.38); 567.88 (+144.19) and 572.51 (+138.28) units of total proteins respectively in their mid gut tissue homogenate. Stevia inulin treatment to the leaves of mulberry, *Morus alba* (L) and feeding them to the fifth instar larvae of silkworm, *Bombyx mori* (L) was found effected into significant changes in the quantity of soluble proteins (32 – 74 percent) and total proteins (5.657– 36.270 percent). Digestibility of the silkworm larvae may be affected through the inulin treatment[12,13].

Table-2: Contents of proteins and activity of enzymes in the mid gut tissue of the fifth instar larvae of silkworm, *Bombyxmori* (L) (Race: PM x CSR2) fed with the aqueous solution of Stevia inulin powder herbal formulation treated leaves of mulberry, *Morus alba* (L) (M-5: variety).

Moiety → Group ↓	Soluble proteins	Total proteins	Protease activity	Amylase activity
Untreated control (0-0)	138.83 (±48.850)	578.43 (±127.51)	1.787 (±0.155)	3.818 (±0.228)
Water treated control (0-I)	134.11 (±61.485)	567.14 (+159.39)	1.798 (+0.469)	3.838 (+0.898)
Water treated control (0-II)	138.07 (±55.667)	568.89 (±144.23)	1.787 (±0.522)	3.838 (±0.898)
Water treated control (0-III)	136.70 (±51.579)	572.51 (+138.28)	1.792 (±0.816)	3.852 (±0.915)
A-1	183.45** (±53.409) 32.147	613.21* (±167.35) 5.657	2.169*** (±0.044) 21.444	4.373* (±0.632) 14.54
A-2	186.29** (±59.932) 33.465	618.16* (±144.78) 6.856	2.237** (±0.148) 25.195	4.466* (±0.872) 16.976
A-3	185.48** (±58.126) 33.595	622.09* (±152.71) 7.185	2.275** (±0.264) 27.323	4.479* (±0.888) 17.317
B-1	219.77** (±99.213) 98.306	684.27* (±299.68) 17.921	2.314*** (±0.278) 29.507	4.558* (±0.915) 19.439
B-2	238.11** (±76.324) 71.439)	759.58* (±122.29) 30.918	2.889*** (±0.349) 61.758	4.843* (±0.928) 26.853
B-3	242.79** (±69.763) 74.162	759.61** (±188.73) 30.923	2.988*** (±0.349) 67.357	4.914** (±0.783) 28.713
C-1	243.47** (±78.296) 74.652	789.42** (±135.18) 36.242	3.027*** (±0.312) 69.428	5.484** (±0.858) 43.646
C-2	243.58** (± 88.137) 74.681	789.64 (±248.26) 36.270	3.088*** (±0.618) 72.62	5.489** (±1.033) 43.804
C-3	242.57** (±88.136) 74.724	789.63** (±313.13) 36.277	3.087*** (±0.983) 72.844	5.588** (±1.132) 46.397
D-1	263.23** (±82.969) 89.605	805.33* (±313.21) 38.986	3.098*** (±0.623) 73.46	5.673** (±0.863) 48.624
D-2	263.47** (±89.712) 89.778	805.39* (±211.98) 38.996	3.118*** (±0.589) 74.58	5.681** (±0.782) 48.834
D-3	263.88** (±87.609) 90.074	805.71* (±262.59) 39.052	3.281*** (±0.616) 83.706	5.888** (±8.823) 54.257

- Each figure is the mean and three replications. - Figure in parenthesis with + sign is the standard deviation. - Figure below parenthesis is percent change. * : P<0.05, ** : P<0.01, *** : P<0.001

The velocity of biochemical reactions catalyzed by the gut-protease enzyme and gut-amylase enzyme in all groups of stevia inulin treatment in the present study was found increased significantly. The percent change in the activity of protease through stevia inulin treatment in the study was 21.444 to 72.62. The percent change in the activity of amylase through stevia inulin treatment in the study was 14.540 to 43.804 and both of they were found significant over the control groups in the present study. Elevation in both fractions of proteins in silkworm larvae received leaves of mulberry processed for the treatment with various concentrations of stevia inulin herbal powder may be explained away through accelerated catalysis / digestion of the contents of food material supplied to the silkworm larvae. Enhanced action of mid gut protease and amylase in silkworm larvae may be effected through feeding stevia inulin treated leaves of mulberry, *Morus alba* (L).

The glycoside terpenoid contents through leaves of mulberry, *Morus alba* (L) are responsible enhanced rate of consumption of food material and utilization of the contents in insects like silkworm, *Bombyx mori* (L). The exogenous compounds entered through the food material in phytophagous insects may be the source of juvenoids. And such exogenous juvenoids are known for enhancement in the rate of synthesis of Polyadenylated RNA or poly (A) RNA. Such type of RNA in silkworm is involved in the synthesis of major silk proteins. Most significant response for stevia inulin treatment to mulberry leaves before feeding to the larval instars of silkworm, *Bombyx mori* (L) in the present study is significant change in quantity of both the protein-fractions and mid gut enzyme activity. The soluble protein contents are known for contribution in the metabolism in the individual tissue through the involvement of enzymes. Applebaum[25] explained the relation between continuous feeding and advancement in the production of enzymes in the mid gut tissue in insects. Accordingly, there is increase in the quantity of mid gut enzymes in the inset involved in continuous feeding. Applebaum[25] concluded this principle stating that, "continuous feeding in insects get reflect into advancement of production of mid gut enzymes". According to the present attempt of study, continuous and qualitative feeding may be responsible for improvement in the qualitative and quantitative efficiency of mid gut enzymes in the fifth stage silkworm larvae.

The last stage larval instars of lepidopteran insects like silkworm, *Bombyx mori* (L) have had four phases of growth. These growth phases: the initial phase of preparation (for the first two days); the phase of accumulation (for the third and fifth days); the phase of regression (for the sixth day) and the phase of degeneration (the day concerned with spinning cocoon around it's body). The initial preparatory phase (for the first two days) is distinguished for synthesis of DNA at significantly higher rate and digestion at significantly higher rate. There is moderate rate of synthesis of RNA and thereby lower rate of synthesis of proteins in this preparatory phase. The phase of accumulation; Silk glands are involved in phase of regression and the phase of degeneration. The significant changes in the quantity and quality of both soluble and total proteins and efficiency of mid gut protease enzyme and mid gut amylase enzyme in the present attempt may be effect stevia inulin on the growth phase of fifth instar larvae of silkworm, *Bombyx mori* (L)[14,15,26].

The stevia inulin treatment for leaves of mulberry, *Morus alba* (L) and supplying for the fifth stage silkworm larvae for first twenty four hours(first day); next twenty four hours (second day) and next twenty four hours (third day) was observed better performance with reference to economic parameters (Cocoon and silk filament). This attempt is as good as to keep silkworm larvae feeding for all their life time, that is to say continuous feeding with improve quality of leaves of mulberry, *Morus alba* (L). Feeding stevia inulin treated continuously for the first three days may be responsible for availing the stevia inulin much more time for interaction with nutrients and the mid gut tissue. That is to say stevia inulin treated mulberry leaves in the alimentary canal are affecting positively on the digestibility in last larval stage in *B. mori.* Further study on other exogenous compounds to treat the leaves before feeding them to the larval instars of silkworm, *Bombyx mori* (L) and to analyse the cocoon characters and silk filament characters should be extended for fortification of the views. The schedule of inulin stevia treatment for the mulberry leaves before supplying larval instars in silkworm, *Bombyx mori* (L) should be…must be.. introduced in the sericulture.

Conclusion

The digestibility in the fifth stage larval stages of *B. mori* correspond to food material ingested. The nutrient contents of herbal formulation: Stevia inulin powder serve for improvement in the ability of digestion. The stevia inulin treated mulberry leaves are responsible for exerting the effect metabolic activities in 5th stage larval in *B. mori*. The stevia inulin treated the mulberry leaves may gear overall biochemical constituency of 5th stage larvae of silkworm, *B. mori*. The method "to treat the leaves of mulberry, *Morus alba* (L) through aqueous solution of stevia inulin before giving them to the larval instars of silkworm, *Bombyx mori* (L)" should be considered for sericultural practices.

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