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EVALUATION OF GROWTH AND BIOCHEMICAL CHANGES IN SILKWORM *BOMBYX MORI* L., VARIETY PM X CSR_2 FED WITH YELLOW LEAVES OF V1 VARIETY OF MULBERRY

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ABSTRACT
India is the second largest producer of silk after China. Hence the scope for increasing the silk production in our country is immense. A large number of varieties of silk worm are used for the silk production. Mulberry belongs to the family Moraceae which has more than 20 species and the common species found in India are, being *Morus alba*, *M. indica*, *M. atropurpurea roxb*, *M. nigra*, *M. serrata* and *M laevigata*. The composition of the leaves varies with variety, degree of maturity and the type of soil in which the plants are grown. Mulberry silkworm is a monophogus insect which feeds only on the mulberry leaves. Growth and development of silkworm and cocoon crop are mainly influenced by yield and nutritional quality of mulberry leaf used as feed. In the present study, the effect of yellow leaves of the mulberry variety V1 on the growth and biochemistry of haemolymph of the silk worm variety PMxCSR2 in three groups *viz*., 3rd to 5th instar, 4th to 5th instar and 5th instar was studied. In the first group, on the 16th day a significant increase in weight of 3.30 ±0.056 g was observed in the control when compared to the treatment which recorded a growth of only 0.27 ±0.016 g. In the second group, growth of only 0.25 ±0.006 g for the treatment and a significantly higher 2.29 ±0.030 g for the control were observed on the 12th day of rearing. In the third group, a significantly higher growth of 2.325 ±0.061 g in the control and only 0.74 ±0.094g in the treatment were recorded on the 9th day. The concentration of proteins and carbohydrates were significantly higher in the control in all the three groups when compared to the treatment while, the concentration of the lipids was significantly higher in the treatment in all the three groups when compared to the control. The concentration of the total proteins and carbohydrates in the yellow mulberry leaves were significantly lower when compared to those of green mulberry leaves confirming the fact that the nutritional value of the mulberry leaves significantly influence the growth and protein, carbohydrate and lipid content of the haemolymph of the silk worm in turn influencing the silk quality and quantity.

KEYWORDS: *Bombyx mori*, Mulberry, yellow leaf, green leaf, haemolymph, growth.

INTRODUCTION

Sericulture is the rearing of silk worms (*Bombyx mori* L) for the production of raw silk and India is the second largest producer of silk after China. Hence the scope for increasing the silk production in our country is immense. A large number of varieties of silk worm are used for the silk production. Mulberry silkworm is a monophogus insect which feeds only on the mulberry leaves. Mulberry belongs to the family Moraceae which has more than 20 species and the common species found in India are, being *Morus alba*, *M. indica*, *M. atropurpurea roxb*, *M. nigra*, *M. serrata* and *M. laevigata*. The composition of the leaves varies with variety, degree of maturity and the type of soil in which the plants are grown (Kalaivani *et al*., 2013). Mulberry (*M. indica* L.) leaf constitutes over 70 per cent of the material required for the biosynthesis of silk proteins. The quality of mulberry leaves has an intimate relation to the healthy growth of larvae and quality of cocoons. Growth and development of silkworm and cocoon crop are mainly influenced by yield and nutritional quality of mulberry leaf used as feed. Proteins and Carbohydrates are the major nutrients responsible for the silkworm growth, development and silk production. Nutritive values of proteins are very important as the silkworm larva utilizes the leaf nitrogenous matter for their growth and development and synthesis of silk protein (Khan *et al*., 2012).

Young green leaves which have attained full size are best suited for feeding silkworm larvae (Koul *et al*., 1994). Nutritive requirement of silkworm larvae vary with the maturity of leaves fed. Chawki silk worms require leaves with high moisture content to digest and late age silkworms require mature leaves with less moisture content since they have the strength to digest mature leaves .On the other hand, too much mature leaves and yellow leaves do not contain sufficient nutrients and hence are not suitable to feed silkworms (Murthy *et al*., 2013). As the larval age increases there is an increase in the level of organic constituent's *viz*., total proteins, total carbohydrates and total lipids. in both control and BmIFV treated batches (Mamatha *et al*., 2014). The protein content of leaves decreases and the carbohydrate, fiber, fat and ash content increases with the maturity of leaves. Young leaves are more acidic than than older ones. It has

been found that accumulation of protein in larvae depends largely on the concentration of carbohydrates in the leaves (Akio *et al*., 1997).

The present study was carried out to evaluate the role of yellow leaves on changes in growth, survival rate and biochemical composition of silk worm fed with yellow leaves using green leaves as control.

MATERIALS & METHODS

For the present study, silk worm *Bombyx mori* race Multi Voltine Hybrid Kolar Gold PM x CSR_2 and the mulberry *Morus indica* variety V1 were selected. The silk worms were reared by following the method suggested by Krishnaswami (1978) at the laboratory of Department of Plant Biotechnology, University of Agricultural Sciences, Bengaluru. Larvae belonging to different stages of the life cycle were selected and divided into 3 batches comprising 1^{st} day of 3^{rd} ,$4^{th}$and 5^{th} instar. Triplicates were maintained in all the treatments with a control. They were all reared till the end of 5^{th} instar and fed daily 3 times.

Evaluation of Growth and survival rate

The silk worm growth performance was evaluated by measuring the weight in grams at a regular interval of 24h in all the treatments including the control. The survival rate was recorded at a regular interval of 24hr from 1^{st} day of 3^{rd}, 4^{th}, 5^{th} instar till the end of 5^{th} instar in all the treatments including the control.

Collection of haemolymph sample for biochemical parameters

The prolegs of silkworm were cut and haemolymph was collected in the eppendorf tubes containing a speck of polythiourea. The collected haemolymph was stored at 5°C for the estimation of different organic constituents.

Estimation of total proteins

The total protein content in the haemolymph was estimated by following the method of Lowry *et al.*(1951). 100 µl of haemolymph and 100 µl of ice-cold 20 % trichloroacetic acid were added in an eppendorf tube and the contents were mixed thoroughly and kept in the refrigerator for 30 minutes. The tubes were centrifuged at 3,000 rpm for 5 minutes. The pellets were washed twice with cold 80 % acetone followed by cold diethyl ether twice. Finally, the pellet was suspended in 500µl of 0.1N NaOH. To 100 µl of this sample, 5 ml of alkaline copper sulphate reagent (To prepare alkaline copper sulphate reagent, Reagent A:2 % Sodium carbonate in 0.1N NaOH; Reagent B: 0.5 % Copper Sulphate and 1 % Potassium Sodium tartrate in 1:1 ratio. Reagent A and B were mixed at 50:1ratio just before the use) was added in a test tube and the contents were mixed well and kept for 30 minutes. Later, 0.5ml of Folin phenol reagent was added to this and the contents were shaken well. After 30 minutes the colour intensity was read at 660 nm in a spectrophotometer against the blank sample which contained 5 ml alkaline copper sulphate reagent and 0.5 ml Folin-phenol reagent. The protein content was recorded from the standard curve prepared for bovine serum albumin (10-100 µg). The protein content was expressed as mg/ml of haemolymph.

Estimation of Total Carbohydrates

The total carbohydrate content in the haemolymph was estimated by the phenol- sulphuric acid method described by Dubois *et al.,*1956.100 µl of haemolymph, 0.4 ml of 5 % phenol were taken in an eppendorf tube and the contents were mixed well. To this 2 ml of conc. H_2SO_4 was added and the contents were mixed well again. The tubes were cooled at room temperature by keeping them in running tap water. The colour intensity was measured at 490 nm in a spectrophotometer against the blank sample b. The total carbohydrate present in each sample was calculated from a standard curve prepared by taking 20-200 µg of glucose. The total carbohydrate present in each sample was expressed as mg/ml.

Estimation of total Lipids

Total lipid content in the haemolymph was estimated gravimetrically following the method of Folch *et al*., 1951. 1ml of haemolymph ,1ml of chloroform:methanol mixture (2:1 v/v) were mixed thoroughly. After 30 minutes, the mixture was centrifuged at 3000 rpm for 15 minutes. The lower chloroform layer containing lipid was collected into pre-weighed plastic vial. To the upper aqueous layer 0.5 ml of chloroform: methanol mixture was added and the procedures was repeated thrice. The pooled chloroform: lipid mixture was air-dried. The vial was weighed to give the lipid content of sample. The quantity of lipid present was expressed in mg/ml of haemolymph.

Estimation of moisture content in yellow and green mulberry leaf

Moisture content in yellow and green mulberry leaves was measured by following method of Khan and Naik (2012). The collected leaf samples were washed in running tap water to remove dust and other residues. Water on the leaf surface was gently removed by using tissue paper, and the weight of leaves was measured and recorded separately for each sample. Samples were dried at room temperature for 24 hrs. followed by oven drying at 60°C for 12 hrs. The leaf weight was measured immediately and recorded separately for each sample. Difference between the weight of fresh leaf and the dry leaf was calculated to give the leaf moisture content.

Estimation of carbohydrate content from leaves

Carbohydrates content in yellow and green mulberry leaves samples was quantitatively measured by Anthrone Reagent method (Dubios, 1956). For estimation of carbohydrates content, 0.2 g of yellow and green leaves were ground separately in distilled water with the help of mortar and pestle. Then leaves samples were centrifuged at 5000 rpm for 10 minute. The clear supernatants were collected in different test tubes and 4 ml of Anthrone reagent was added to obtain green colour. The absorbance was measured by using U-V Spectrophotometer at 625 nm wavelength. The carbohydrates content was calculated by from the standard curve using Dextrose (L) as standard.

Estimation of protein content from yellow and green mulberry leaves

Protein content was quantitatively measured by Lowry's method (1951). About 0.5 gm of yellow and green mulberry leaf samples were washed well to remove surface dust and other residues. Then the leaf samples were crushed and ground in 5 ml of Trichloroacetic acid solution (T.C.A.). The ground material was collected in centrifugation tubes and centrifuged at 4000 rpm for 15 minutes. The clear supernatant was collected and assayed for protein content by addition of Folin's reagent. The absorbance of blue color was measured with the help of U.V. Spectrophotometer at 650 nm wavelength. The protein content was calculated by using the standard curve and Bovine Serum Albumin was used as standard.

Statistical Analysis

The data obtained were statistically analyzed by t-Test and one way ANOVA using SPSS to determine the significant difference among and between the treatments and the control.

RESULTS & DISCUSSIION

Growth Performance: The growth performance of the three batches of silk worms both in the treatment and the control is presented in table 1. In the 3rd-5th Instar group significant difference in the growth was observed from 9th day onwards between the treatment and the control. However, no significant difference in growth was observed within the treatment means up to 16th day. On the 16th day a significant increase in weight of 3.30 ±0.056 g was observed in the control when compared to the treatment which recorded a growth of only 0.27 ±0.016 g. Even in the second group, there was no significant difference in growth within the treatment up to 12th day. However, there was a significant difference in growth between the treatment and the control from 5th day onwards with only 0.25 ±0.006 g for the treatment and a significantly higher 2.29 ±0.030 g for the control on the 12th day of rearing. In the third group, there was no significant difference between the treatment and the control up to 5th day. From 5th day onwards there was a significant difference between the treatment and control up to 9th day with a significantly higher growth of 2.325 ±0.061 g in the control and only 0.74 ±0.094g in the treatment on the 9th day (Table 1).

TABLE 1: Weight of silk worms in the three groups fed with yellow and green mulberry leaves

Number of days	Weight of Silk Worm (in gms)					
	3rd to 5th Instar		4th to 5th Instar		5th Instar	
	Treatment	Control	Treatment	Control	Treatment	Control
1 days	0.03±0.001 a*	0.06±0.015 a	0.10±0.002 a	0.12±0.002 a	0.12±0.008 a	0.12± 0.005a
2 days	0.04±0.002 a	0.06±0.001 a	0.14±0.004 a	0.25±0.006 a	0.15±0.013 a	0.20±0.009 a
3 days	0.07±0.015 a	0.13±0.029 a	0.14±0.005 a	0.43±0.010 a	0.16±0.011 a	0.30±0.012 a
4 days	0.07±0.002 a	0.19±0.002 a	0.18±0.009 a	0.52±0.012 a	0.20±0.010 a	0.45±0.015 a
5 days	0.09±0.024 a	0.13±0.002 a	0.19±0.007 a	0.66±0.010 b	0.25±0.014 a	0.62±0.028 b
6 days	0.08±0.002 a	0.22±0.007 a	0.22±0.009 a	0.92±0.035 b	0.51±0.062 a	1.32± 0.040c
7 days	0.14±0.031a	0.40±0.011 a	0.23±0.007 a	1.39±0.019 c	0.66±0.087 b	1.70± 0.043 c
8 days	0.10±0.004 a	0.51±0.011 a	0.25±0.007 a	1.67±0.032 c	0.69±0.091 b	2.27±0.040 c
9 days	0.12±0.004 a	0.61±0.011b	0.23±0.008 a	1.98±0.051 c	0.74±0.094 b	2.325±0.061 c
10 days	0.13±0.016 a	0.72±0.015 b	0.25±0.006 a	2.28±0.029 c		
11 days	0.19±0.034 a	1.19±0.044 b	0.24±0.006 a	2.61±0.057 c		
12 days	0.14±0.009 a	1.56±0.043 c	0.25±0.006 a	2.29±0.030 c		
13 days	0.24±0.063 a	1.80±0.053 c				
14 days	0.19±0.012 a	2.32±0.052 c				
15 days	0.22±0.014 a	2.50±0.069 c				
16 days	0.27±0.016 a	3.30±0.056 c				

*Values with same superscripts are not significantly different

Protein concentration in the haemolymph: The total protein content in the haemolymph increased significantly as the age of the larvae increased both in control and treatment in all the three groups *viz*., 3rd to 5th instar , 4th to 5th instar, 5th instar (Table 2,3 & 4,).

TABLE 2: Concentraion of the total proteins, carbohydrates and lipids in the haemolymph of 3rd to 5th instar silk worm

Number of days	Total Proteins (in mg/ml)			Total carbohydrates (in mg/ml)			Total lipids (in mg/ml)		
	Treatment	Control	't' value	Treatment	Control	't' value	Treatment	Control	't' value
1 day	5.30±0.01	5.31±0.01	1.22	6.3±0.005	6.31±0.01	1.22	8.3	8.31	1.224
2 days	6.04±0.01	6.62±0.17	3.39*	5.17±0.128	6.62±0.17	6.76**	11.16	10.04	106.569**
3 days	6.16±0.01	7.06±0.01	61.71**	5.16±0.008	6.06±0.01	61.71**	14.06	12.38	115.396**
4 days	6.30±0.05	7.31±0.05	23.69**	6.18±0.005	7.18±0.01	122.47**	15.88	13.85	50.141**
5 days	7.04±0.01	8.62±0.17	9.21**	6.21±0.012	7.76±0.01	103.75**	19.39	18.11	85.865**
6 days	7.17±0.01	12.06±0.11	320.12**	6.3±0.057	7.84±0.00	26.74**	20.56	18.61	145.75**
7 days	7.41±0.03	19.75±0.00	362.91**	6.32±0.011	8.92±0.01	201.39**	24.51	22.13	103.057**
8 days	7.68±0.03	22.23±0.05	218.2**	6.42±0.011	8.96±0.12	152.2**	27.67	23.56	103.057**
9 days	8.05±0.03	17.41±0.01	362.51**	6.25±0.015	8.97±0.15	125.91**	24.17	21.11	205.495**
10 days	8.90±0.04	29.43±0.01	465.42**	6.32±0.046	8.98±0.00	57.29**	25.56	22.44	259.322**
11 days	8.74±0.02	33.29±0.08	111.31**	6.52±0.011	9.14±0.17	124.59**	28.56	24.61	232.398**
12 days	8.85±0.02	36.06±0.08	153.04**	6.44±0.017	9.35±0.02	116.52**	30.94	27.54	141.587**
13 days	8.87±0.03	40.53±0.05	134.98**	6.36±0.037	9.47±0.02	72.12**	35.03	28.77	383.345**
14 days	8.41±0.08	41.23±0.01	201.63**	6.26±0.011	9.56±0.01	177.98**	35.06	27.06	848.528**
15 days	8.22±0.17	43.41±0.01	168.72**	6.14±0.005	9.68±0.11	274.20**	38.06	28.11	585.407**
16 days	8.46±0.02	44.16±0.03	981.69**	6.06±0.031	9.80±0.01	110.11**	37.9	27.40	33.258**

NS: Non Significant; *Significant at 5% level; **Significant at 1% level

TABLE 3: Concentraion of the total proteins, carbohydrates and lipids in the haemolymph of 4th to 5th instar silk worm

Number of days	Total Protein			Total carbohydrate			Total lipid		
	Treatment	Control	't' value	Treatment	Control	't' value	Treatment	Control	't' value
1 day	6.3±0.005	6.31±0.005	1.224**	8.18±0.005	8.18±0.005	-------	9.85 ±0.020	9.85 ±0.020	-----
2 days	7.04±0.005	7.95±0.161	5.665**	7.21±0.012	8.76±0.008	103.753**	20.39±0.012	19.11±0.008	85.860**
3 days	8.16±0.008	12.06±0.011	268.18**	7.3±0.057	8.84±0.003	26.744**	21.56±0.005	19.61±0.012	145.75**
4 days	11.38±0.00	19.75±0.008	671.09**	7.32±0.011	8.91±0.005	123.160**	25.51±0.015	23.13±0.017	103.057**
5 days	19.68±0.00	22.23±0.005	312.30**	7.42±0.011	8.95±0.012	92.2**	28.67±0.011	25.56±0.005	240.899**
6 days	23.03±0.00	27.41±0.005	291.48**	7.25±0.015	8.97±0.015	79.620**	25.17±0.012	22.11±0.008	205.494**
7 days	24.94±0.01	29.43±0.005	347.79**	7.32±0.046	8.98±0.003	35.754**	26.56±0.011	23.44±0.003	259.322**
8 days	25.82±0.00	33.29±0.008	603.39**	7.52±0.011	9.14±0.017	77.159**	29.56±0.014	25.61±0.008	232.397**
9 days	26.82±0.00	36.06±0.008	740.84**	7.44±0.017	9.35±0.017	76.436**	31.94±0.021	28.54±0.01	141.587**
10 days	25.84±0.00	40.53±0.005	1393.3**	7.36±0.037	9.47±0.021	48.905**	36.03±0.005	29.01±0.656	10.546**
11 days	29.41±0.00	41.23±0.012	792.90**	7.26±0.011	9.56±0.014	124.107**	36.06±0.008	28.06±0.003	848.528**
12 days	24.22±0.01	43.41±0.012	899.09**	7.14±0.005	9.68±0.011	196.747**	39.06±0.012	29.11±0.012	585.406**
13 days	22.45±0.01	44.16±0.027	668.21**	7.06±0.031	9.80±0.012	80.701**	38.56±0.018	28.40±0.017	396.815**

NS: Non Significant; *Significant at 5% level; **Significant at 1% level

TABLE 4: Concentraion of the total proteins, carbohydrates and lipids in the haemolymph of 5th instar silk worm

Number of days	Total Protein			Total carbohydrate			Total lipid		
	Treatment	Control	't' value	Treatment	Control	't' value	Treatment	Control	't' value
1 day	18.03±0.005	18.41±0.005	46.540**	8.97±0.015	8.97±0.015	0	20.113±0.008	20.113±0.008	0
2 days	25.94±0.011	28.43±0.005	192.874**	7.32±0.046	8.98±0.003	35.754**	24.56±0.001	23.443±0.003	92.912**
3 days	28.82±0.008	32.29±0.008	280.248**	7.52±0.011	9.14±0.017	77.159**	25.613±0.014	25.6133±0.008	55.893**
4 days	29.82±0.008	35.06±0.008	420.134**	8.44±0.017	9.35±0.017	36.347**	28.943±0.021	26.54±0.01	99.984**
5 days	31.84±0.008	39.53±0.020	729.221**	8.36±0.037	9.47±0.021	25.690**	30.03±0.005	28.77±0.015	77.158**
6 days	37.41±0.008	41.23±0.012	256.253**	8.26±0.011	9.56±0.014	70.225**	32.063±0.008	29.063±0.003	318.198**
7 days	39.22±0.017	42.41±0.012	149.458**	8.14±0.005	9.68±0.011	119.287**	33.066±0.012	28.116±0.012	291.232**
8 days	40.45±0.017	44.16±0.027	114.191**	8.06±0.031	9.80±0.012	51.284**	32.566±0.018	27.406±0.017	201.532**

NS: Non Significant; *Significant at 5% level; **Significant at 1% level.

In the first group, on the 16th day, a significantly higher protein concentration of 44.16±0.03 mg/ml in the control was recorded when compared to 8.46±0.02 mg/ml in the treatment. In the second group, a significantly higher protein concentration of 44.16±0.027 mg/ml in the control was observed when compared to only 22.45±0.01 mg/ml in the treatment on the 13th day of rearing. In the third group also there was a significant increase in the protein concentration of 44.16±0.027 mg/ml in the control when compared to only 40.45±0.017 mg/ml in the treatment on the 8th day. On the other hand in yellow leaves fed haemolymph total protein content was 5.31 mg/ml, 6.31 mg/ml & 18.03 mg/ml and was increased to 8.46 mg/ml, 22.45 mg/ml, 40.45 mg/ml during last day of treatment in all 3batches respectively. There was a significant reduction in total protein content yellow leaves fed silkworm haemolymph compared to control in all the batches during respective treatment. The percent reduction in total protein content was ranging from 0.19 to 80.84%, 0.16 to 49.16% and 2.06 to 8.40 % in the 1st, 2nd and 3rd groups respectively. The highest percentage of decrease in protein concentration of the haemolymph of 80.84% was observed in the 1st group and the lowest percentage of decrease of 8.40% was recorded in the 3rd group (Fig. 1).

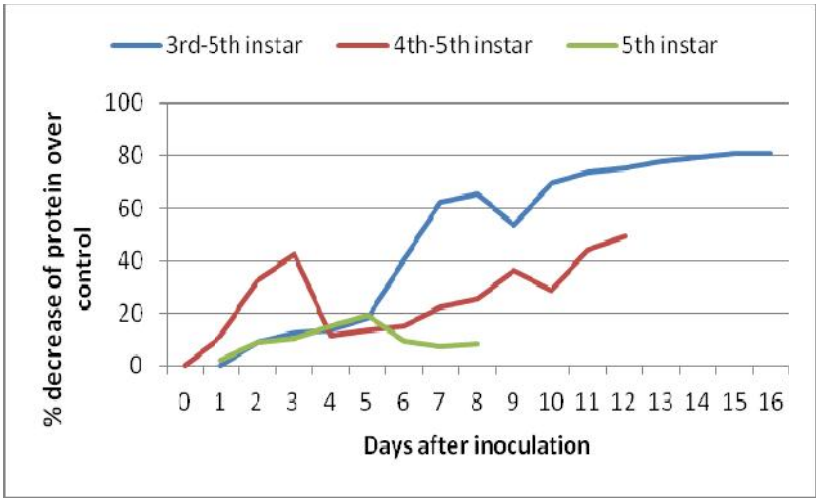

FIGURE 1: Percentage decrease of total proteins in the treatment over the control in the heamolymph of silk worm.

Carbohydrate concentration of the haemolymph

In control, total carbohydrate was ranging from 6.31 mg/ml to 9.80mg/ml, 8.18 to 9.80 mg/ml , 8.97mg/ml to 9.80 mg/ml from 1st day of treatment to end of treatment in all 3 batches respectively. While in the treatment, total carbohydrate increased from 5.17 to 6.52mg/ml and afterwards decreased to 6.06mg/ml at the end of treatment in 3rd to 5th instar. While in 4th to 5th instar, it increased from 7.21 mg/ml to 7.52 mg/ml and then decreased to 7.06 mg/ml at the end of instar. Similarly in 5th instar treated larvae, total carbohydrate increased from 7.32 mg/ml to 8.36 mg/ml and then decreased to 8.06 mg/ml at the end of treatment.

It was observed that the total carbohydrate content was reduced in the treatment when compared to respective controls in all the batches (Table 2, 3 & 4). The percent of reduction in total carbohydrate content in treatment when compared to control ranged from 0.16 to 38.16%, 17.70 to 27.96 % and 18.48% to 17.75% in the 1st , 2nd and 3rd group respectively (Fig. 2).

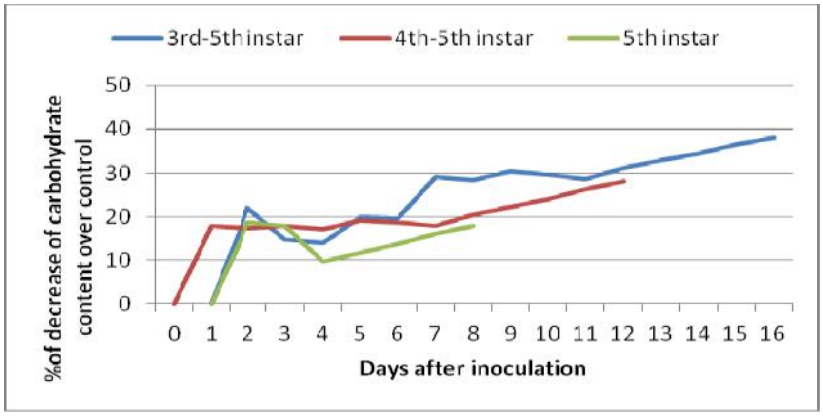

FIGURE 2: Percentage decrease of total carbohydrates in the treatment over the control in the heamolymph of silk worm

Lipid concentration of the haemolymph

The total lipid content increased in both control and treatment batches (Table 2, 3 & 4). The total lipid content in treated batches ranged from 8.3 mg/ml to 37.9 mg/ml, 9.85 mg/ml to 38.56 mg/ml and 20.113 mg/ to 32.506 mg/ml in 1st, 2nd and 3rd groups respectively. The total lipid content increased significantly in treated larvae compared to respective controls. The percentage of lipid increased in treated larvae compared to control and ranged from 10.03 to 27.70%, 6.28 to 26.35 % & 4.55 to 15.84% in 3rd to 5th, 4th to 5th& 5th instar larvae respectively (Fig. 3).

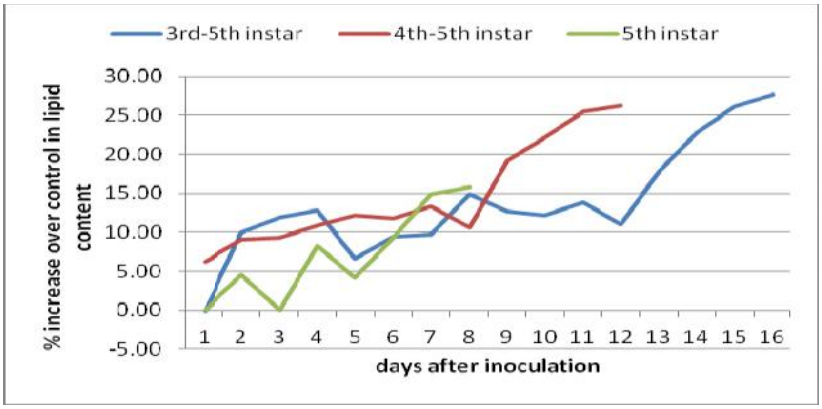

FIGURE 3: Percentage increase of total lipids in the treatment over the control in the heamolymph of silk worm

Reduction in growth rate and haemolymph concentration of proteins, carbohydrates and lipids of silkworm fed with yellow leaves were also reported in the earlier studies of Siraj *et al*., 2007 in CSR2 silk worm breed. The decrease in total protein content in haemolymph and growth rate can be attributed to the disintegration of structural organization of sub-cellular level and also by activated proteolysis or impaired protein synthesis in the tissues. The growth and development of larvae always depends on active synthesis of protein in the tissues (Engemann, 1965; Tazima, 1978.) The decrease in protein content during the BmIFV infection therefore could have inhibited the growth of larval development. It is evident from the stunted growth of the larvae observed during BmIFV infection in susceptible breed. There was a significant depletion in total carbohydrate content in haemolymph during the progressive infection of BmIFV treated larvae compared to control. Carbohydrates serve as main source of energy to a number of insect species (Chino and Gilbert, 1965) and in biological system. The amount of lipid in the haemolymph and midgut tissue increased markedly during the progressive infection of BmIFV. The marked increase in the lipid content of virus infected larvae had been attributed to the altered host of lipid metabolism (Kamano *et al.,* 1966). The results of the present study are in agreement with the earlier results. The larvae became inactive and under-sized larvae were noticed during the rearing period. As the larval age

increases, the disease symptoms become more discernible and diseased larvae became dull, soft and flaccid before death and developed dysentery and vomited gut juice. These symptoms are also similar to the earlier reports of Sanakal *et al.*, 1966 and Mamatha *et al*., 2014.

Moisture, Protein and Carbohydrate Concentration in the Mulberry Leaves

Moisture , protein and Carbohydrate content was higher in green leaves compare to yellow leaves in all the three seasons that is rainy, winter and summer (Table 5).

TABLE 5: Biochemical composition of yellow and green leaves of the Mulberry

Season	Mulberry leaves	Proteins (mg/gm)	Carbohydrate (mg/gm)	Moisture (%)
Rainy	Green leaf	223.26	38.60	80.64
	Yellow leaf	98.08	20.08	66.72
Winter	Green leaf	221.40	37.50	78.19
	Yellow leaf	92.82	21.82	64.77
Summer	Green leaf	215.07	34.68	75.73
	Yellow leaf	88.92	19.91	63.16

However, moisture, protein and carbohydrate content was higher in rainy season both in green and yellow leaves. Khan and Naik (2012) also observed higher percentage of proteins, carbohydrates and moisture content in the V1variety of green mulberry leaves. In leaf, moisture content plays a vital role in improving nutrition levels which in turn improve the palatability and digestibility of leaves by silkworms as well as normal growth and development of silkworms and cocoon quality (Koul *et al*., 1996). Availability of moisture content in leaves enhances feeding efficiency of silkworm larvae which in turn increases growth rate (Sastry *et al*., 1988). Decrease in leaf moisture content influenced different energetic parameters such as assimilation and conversion efficiency of food which decreases with decreasing dietary moisture content of leaf. Leaves with high moisture remain fresh and acceptable to silkworm. Due to some diseases and some pests (Nematodes and bacterial leaf scorch) the green leaves turn yellow and silk worms feeding on these yellow leaves, have lesser growth rate, protein content, carbohydrates contents and also exhibit low survival rate.

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