

Impact of turmeric on the protein and lipid metabolic profiles of silkworm, *Bombyx mori* L. and cocoon production

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ABSTRACT

Sericulture industry has played an important role in the economic life of man ever since its discovery (4000 years). It is being presently practiced in about 42 countries in the World. China and India occupies the first and second positions in the production of raw silk in the World. Mulberry cultivation, silkworm rearing and silk reeling are the three major components in the silk industry. Mulberry, being the only food plant for the silkworm, *Bombyx mori*., disease free mulberry leaf with high nutritive value and improved strains of silkworm have been considered as the two prime aspects for the development of this silk industry. The phenomenal health benefits of turmeric (*Curcuma longa*) were being explored since several hundreds of years. There's still a vast untouched potential on the miracle working power of curcumin in turmeric. In this context, mulberry leaves were treated with different concentrations of turmeric to check for its positive effect. This piece of study evaluated for the potential effect of Curcumin on protein and lipid metabolisms of the silkworm, *Bombyx mori*, the key enzymes involved in these metabolisms and treated batches are further taken up to study the energy metabolism through the levels of ATPase and Cytochrome-C activity levels. This study also tried to assess the state of cocoon yield from the turmeric fed *Bombyx mori* larvae. Throughout the study, the *Bombyx mori* larvae and cocoons were found to be disease free.

KEY WORDS: *Bombyx mori* L., cocoon yield, *Curcuma longa*, *Morus alba*

INTRODUCTION

Silk mesmerizes everyone by its customs, rich and royal touch, it imparts into one's mind. In the global silk production, India occupies the coveted second place. For our country, the Sericulture based activities rightly fit into the socio economic structure of our rural scenario and works as an effective tool for rural reconstruction to uplift the economy of down trodden sections of rural populace. Sericulture is spread over nearly 60,000 villages throughout the subcontinent but mostly concentrated in

South India. Though, Indian sericulture was striving to evolve new races, the stable strains could be established only with the introduction of what is called 'New technology' of silkworm cocoon production. India is now in the threshold of producing international grade silk through this new technologies. Success of silk processing depends on quality of silk cocoons, which forms an integral part of raw silk production in sericulture. To harvest quality cocoons, disease free, nutritive mulberry leaf and improved strains of silkworm are primarily most important inputs to sustain this industry.

To this end, the impact of turmeric has been tried in this study, on silkworm to check for its efficacy on increasing the cocoon yield.

The rhizome of *Curcuma longa* L (Family: Zingiberaceae) named turmeric is the perennial herb. The major constituents being curcumin, also contains curcuminoids, fats, minerals, fibers, vitamins, proteins and carbohydrates. Apart from being used in almost all Asian kitchens, it has also been used in indigenous systems of medicine in Asia during the past three decades. The curcumin (Diferuloyl methane) is one of the most active ingredients, which is responsible for its biological activity; antimicrobial (Lutomeski *et al.*, 1974), antileishmanial (Gomez *et al.*, 2002), Lepato protective (Kiso *et al.*, 1983), Anticancer (Kutton *et al.*, 1985). Curcumin has also been tried as a possible immune system stimulator (Agarwal *et al.*, 2006, Jagetia *et al.*, 2007) which modulates the activation of T-cells, B-cells, Macrophages, Neutrophils, Natural killer cells and Dendritic cells; and down regulates various proinflammatory cytokines and chemokines and enhance antibody responses M.D. Anderson Cancer centre, Texas (2007) researches also reported the curcumin's role in modulating the immune system. However, this has not been tried either as mulberry fortifying agent or silkworm health supplement for higher cocoon yield. Hence, the present study has been taken up to study the effects of turmeric on silkworm metabolism and cocoon production

MATERIALS AND METHODS

The study was carried out in the Division of Sericulture, Tirupati, A. P., India. Fine turmeric powder (*Curcuma longa*) commercially available was selected for the study. All other chemicals

used were of technical grade from Sigma, St. Louis, USA, SDH, CDH (India).

A hybrid race PM X NB₄D₂ (Cross breed) of the silkworm *Bombyx mori* was used. The standard silkworm rearing method was followed, described by the Krishanswami (1978a). Mulberry constitutes the sole and satisfactory food of the silkworm *Bombyx mori* and mulberry cultivation is indispensable for silkworm rearing.

Biochemical analysis:

Total Proteins were estimated by using crystalline bovine serum albumin as the standard. The protein content was expressed as mg/g wet weight of the tissue or 1 ml of haemolymph.

The total FAA levels in control and experimental tissues and haemolymph of *Bombyx mori* larva were determined by the method of Moore and Stein (1954). The values were expressed as μM of tyrosine/gm. wet weight or 1 ml of haemolymph.

Protease activity was estimated by the method of Moore and Stein (1954), with 5% homogenate at 10000 rpm for 15 minutes. The proteolytic activity is expressed as μM of tyrosine /mg. protein/hr or 1ml of haemolymph.

Serum transaminases:

In the colorimetric method, these enzymes catalyse the transfer of α -amino groups from specific amino acids to α -ketoglutaric acid to yield glutamic acid and oxalo acetic acid or pyruvic acid. The formed ketoacids were then determined colorimetrically by the method of Reitman and Frankel (1957).

Estimation of Aspartate Amino Transferase (AAT) (L-aspartate, 2-oxoglutarate amino transferase EC 2.6.1.1) activity:

0.2 ml of the homogenate was pipetted into a clean test tube. To this, 100 μ M of L-aspartate, 100 μ M of phosphate buffer (pH 7.5) and 2 μ M of α -ketoglutaric acid were added. The reaction was carried out at 37° for 30 minutes. After incubation, 0.5 ml of 2, 4-DNPH was added to arrest the reaction. After keeping the tubes for 20 minutes at room temperature, added 5 ml NaOH (1N) and mixed thoroughly. The colour developed was read in a spectrophotometer at 505 nm against the reagent blank. Zero time controls were maintained. The colour intensity was proportional to the transaminase activity and was expressed as μ M of pyruvate formed/mg. protein/hr or 1 ml of haemolymph.

Assay of anine amino transaminase (ALAT) (DL- alanine; 2-Oxaloglutarate Amino Transferase E C: 2.6.1.2) activity:

3% homogenates of control and experimental 4th and 5th instar *B.mori* larval intestine, silk gland, muscle and haemolymph were prepared in phosphate buffer and centrifuged at 1500 rpm for 10 min. 0.2 ml of the supernatant was pipetted into a clean test tube. To this 100 μ M of L-alanine, 100 μ M of phosphate buffer (pH 7.5) and 2 μ M of α -ketoglutaric acid were added. Then the reaction was carried out at 37°C for 30 minutes. After incubation, 0.5 ml of 2, 4-dinitrophenyl hydrazine hydrochloride (DNPH) was added to arrest the reaction. After keeping the tubes for 20 minutes at room temperature, added 5 ml of NaOH (1N) and mixed thoroughly. The colour developed was read at 505 nm against a reagent blank in a spectrophotometer. Zero-time controls were also maintained. The colour intensity was

proportional to the transaminase activity and was expressed as μ M of pyruvate formed/mg protein/hr or 1 ml of haemolymph.

Measurement of glutamate dehydrogenase activity (GDH) (L-glutamate: NAD oxidoreductase, EC1.4.1.3):

The GDH activity was assayed by the method of Lee and Lardy (1965). The GDH activity was expressed as μ moles of formazan per mg protein per hour or 1 ml of haemolymph.

Estimation of Free Fatty Acids (FFA):

Lipids in control and experimental tissues and haemolymph were extracted in chloroform: methanol mixture (2:1 ratio) and FFA present were determined by suitably modifying the procedure of Schmidt *et al.* (1979) as given by Berg-Mayer (1974). The values of free fatty acids were expressed as μ M of stearic acid/gm. wet weight of the tissue or 1 ml of haemolymph.

Determination of Lipase Activity (Lipase esterase E.C. 3.11.3):

The activity levels of lipase were estimated by the method of Bier (1957). The lipase specific activity was expressed as μ moles of paraphenol formed/mg protein / hr or 1 ml haemolymph.

Determination of ATPases activity (ATP phosphohydrolase: EC.3.6.1.3):

Total ATPase activity was measured by the method of Fritz and Hamrick (1966) and as modified by Desai and Ho (1979).

Total ATPases:

Total ATPase activity in control and experimental intestine, silk gland muscle tissues and haemolymph of 4th and 5th instar larvae was assayed by measuring the inorganic phosphate (pi) liberated from hydrolysis of ATP.

Cytochrome-C-Oxidase(cyt-C-oxidase) activity (Ferrocyclochrome-C-Oxygen Oxidoreductase EC: 1.9.3.1)

In control and experimental tissues of intestine, silk gland, muscle and haemolymph cytochrome-c-oxidase activity was measured. The enzyme activity was expressed in μg of formazan formed/mg protein/hr or 1 ml of haemolymph.

Quantitative parameters of cocoons:

The quantitative parameters of cocoons viz., cocoon weight, shell weight, shell percentage, filament length and denier were determined by the methods given by Sonwalker (1993).

Cocoon weight:

A sample is drawn from each replication comprising around 20-25 cocoons, representing the entire quality of each replication. Individual cocoon weights were taken from each sample and the mean cocoon weight was found respectively for treatment and control batches. The weights were taken in gram units.

Shell weight and Shell percentage:

The shell weight and shell percentages are important economic characters. The shell ratio depends upon the silkworm race and is normally higher in male than in female. Shell percentage varies according to the variation in cocoon weight caused due to the loss of moisture present in the pupae. The weights were taken in gram units. Shell percentage = $\frac{\text{Weight of the cocoon shell}}{\text{Weight of the entire cocoon}} \times 100$.

Filament length:

The length of the silk filament was measured by subjecting the single cocoon to reeling in a mono-cocoon reeling machine called Eupprouvette. The total filament length was calculated as follows

(Sonwalker, 1993). Total filament length in mts = No. of rotations x Circumference of the reel.

Denier:

The filament size was calculated for control and experimental batches by using the formulae as given by Sonwalker (1993). Variation in the denier of the cocoon silk filament will ultimately determine the uniformity and quality raw silk yarn reeled. Variations in thickness of the individual filament influence the size or thickness of the raw silk yarn. Denier = $\frac{\text{Weight of the filament}}{\text{Length of the filament}} \times 9000$.

RESULTS

Total proteins:

In the 4th and 5th instar 1% turmeric caused more percent elevation of larval tissue total proteins and was followed by 10% > 100 %. The increase in total protein content was found to be statistically significant for 1% and 10% turmeric fed larval tissues and haemolymph, over the control ($p < 0.001$) in both 4th and 5th instar *B. mori* larvae (Table-1).

Free amino acids:

Turmeric sprayed mulberry leaf fed *Bombyx mori* larval tissues showed enhanced levels of their FAA contents was presented in table-2, and the changes were found to be statistically significant over the control ($p < 0.001$) in both 4th and 5th instar larvae (Table-2).

Protease activity:

The intestine, silk gland, haemolymph and muscle tissues from turmeric fed 4th and 5th instar larvae showed elevated levels of their protease activity over the control and the changes were found to be statistically significant ($P < 0.001$) over the control (Table-3).

AAT and ALAT:

The turmeric sprayed mulberry leaf fed 4th and 5th instar *Bombyx mori* larval intestine, silk gland, haemolymph and muscle tissues receiving 1 % & 10 % turmeric showed statistically ($P < 0.001$) increased levels of their AAT and ALAT activity levels over the control. (Table-4&5).

Glutamate dehydrogenase:

The intestine, silk gland, haemolymph and muscle from turmeric fed 4th and 5th instar *Bombyx mori* larvae showed increased levels of their GDH activity (Table-6) and the changes were found to be statistically significant over the control in both 1 % and 10 % turmeric fed larvae ($P < 0.001$).

Total lipids:

Turmeric fed 4th and 5th instar larval tissues and haemolymph showed decreased levels (Table- 7) of their total lipid content and the changes were found to be statistically significant over the control ($P < 0.001$).

Free fatty acids (FFA):

Turmeric sprayed mulberry leaf fed 4th and 5th instar *Bombyx mori* larval tissues and haemolymph showed enhanced levels of their FFA content and the changes were found to be statistically significant over the control (Table-8).

Lipase activity:

Turmeric sprayed leaf fed larval intestine, silk gland, haemolymph and muscle lipase activity showed enhanced levels and the changes were found to be statistically significant over the control. More percent elevation of tissue and haemolymph lipase activity were observed in 1% turmeric fed larval tissue and

haemolymph (Table-9) and was followed by 10% > 100% turmeric.

Total ATPase activity:

The data in Table 10 shows the control and turmeric sprayed mulberry leaf fed 4th and 5th instar *Bombyx mori* larval intestine, silk gland, haemolymph and muscle total ATPase activity levels. Turmeric treatment significantly ($p < 0.001$) increased the *Bombyx mori* larval tissues and haemolymph total ATPase activity levels.

Cytochrome-c oxidase activity:

Turmeric sprayed mulberry leaf fed *Bombyx mori* larval intestine, silk gland, haemolymph and muscle showed enhanced cytochrome-c oxidase activity levels (Table-11) and all the changes were found to be statistically significant over the control ($p < 0.001$).

Cocoon weight:

Cocoons from 1% turmeric fed larvae showed increased Cocoon weight and the percent increase was found to be 12.54% over the control and was followed by 10% (7.23%) > 100% (-2.62%) (Table-12). Cocoons from 5th instar (Table-13) larvae showed higher percent elevation over the control compared to the Cocoon weight from turmeric fed 4th instar larvae (Table-12).

Shell weight:

Cocoons from 1% turmeric fed larvae showed more percent increase of their shell weight; 24.50% and 39.92% in cocoons from 4th and 5th instar turmeric fed larvae respectively (Table 12 & 13) and was followed by 10% > 100%.

Shell percentage:

More shell percentage was recorded in the cocoons from 1% turmeric fed 4th and 5th instar *B. mori* larvae (14.39% and 18.79% respectively) and the

increase in shell percentage was found to be statistically significant (Table 12 & 13) over the control ($p < 0.001$).

Filament length:

From the data in table 12 and 13, it is evident that the silk from 1% turmeric fed *B. mori* larval cocoons showed increased filament length over the control ($P < 0.001$) compared to 10% and 100% turmeric concentration.

Denier:

The data in table 12 and 13 further depicts that the silk from 1% turmeric fed 4th and 5th instar *B. mori* larvae showed decreased denier (2.05 and 2.35 and 1% respectively) and the trends were followed by 10% > 100% turmeric.

DISCUSSION

The increase in the body weight of silkworm was accompanied by the accumulation of various biochemical constituents like protein, FAA and enzymes like AAT and ALAT etc (Lang *et al.*, 1965; Church and Robertson, 1966; Kotby *et al.*, 1987; Dhinakar, 1988; Harihara Raju, 2001; Mamatha, *et al.*, 2002). Tissue proteins undergo a continuous process of renewal, referred to as "turnover". Biochemical constituents like total and soluble proteins, FAA, ALAT and AAT, protease activity have been examined in tissues of silkworm with reference to its metamorphosis. The levels of amino acids show variations during metamorphosis and under stress conditions of the *Bombyx mori* (Harihara Raju *et al.*, 2002; Mamatha *et al.*, 2002). Amino acids are the most important constituents in silkworm nutrition (Bose *et al.*, 1989).

The major transmission reactions are performed by alanine aminotransferase (Lehninger, 1993). Alanine aminotransferase recorded a higher

activity compared to aspartate aminotransferase all through the embryonic development in silkworms (Pant and Kumar, 1979). Proteases are the most commonly found digestive enzymes in insects (Ann Sorensen *et al.*, 1983). Several factors responsible for the secretion of the proteolytic enzymes have been investigated by (Briegel and Lee, 1975; Mordue, 1967; Eguchi *et al.*, 1972; Sarangi *et al.* 1986). The total proteins (table-1) and free amino acid contents (table-2) were measured in the different tissues of both control and turmeric sprayed mulberry leaf fed larvae, with lowering percentage (1%) of turmeric an increase in both total protein and free amino acid contents were observed and the trend was followed by 10% > 100% fed larvae.

Elevation in the proteins in turmeric sprayed mulberry leaf fed larvae is an indication of high protein synthesis in *Bombyx mori* larvae. The lower the turmeric concentration the higher the protein and amino acid synthesis was observed. So different doses of turmeric may affect the protein synthesizing capacity of the larvae. The levels of free amino acids were estimated in both control and turmeric treated mulberry leaf fed larvae. Silkworm and other insects are known to contain usually large amount of free amino acids (Sinha *et al.*, 1990; 1991). The larvae are metabolically more active as evidenced by the presence of increased levels of proteins and total free amino acids under turmeric stress. Increase in turmeric fed *Bombyx mori* larval tissue protease activity (table-3) reflect a state of breakdown of proteins resulting in the formation of total FAA which were recorded as increased in turmeric fed *Bombyx mori* larval tissues (table-2).

From the current study, it is evident that both transaminases are active in the control tissues. Based on the results

of table 4&5 it can safely be concluded that the above cited situation is pronounced more in 1 % turmeric fed larval tissues and less to the other concentrations of turmeric fed *B.mori* larval tissues and haemolymph. AAT at any time represent channeling of FAA directly into TCA cycle thus enhancing energy yield through aerobic metabolism.

The turmeric fed *Bombyx mori* larval tissues showed elevated activity levels of their GDH (table-6). Glutamate dehydrogenase is an enzyme of great importance in the intermediary metabolism of amino acids. Glutamate and GDH have a unique role in amino group transfer. It is through this enzyme that α -ketoglutarate is made available for the citric acid cycle, at the same time from ATP to release ammonia. The enhanced activity of GDH in the *Bombyx mori* larval tissues under turmeric stress indicates increased oxidation of glutamate (Sailaja, 1999). The role of α -ketoglutarate as a substrate for sperm mobility was demonstrated (Osanai *et al*, 1986, 1987a&b) in the silkworm *Bombyx mori*.

Total lipids were analyzed during developmental stages in silkworms such as *Bombyx mori* (Nakasone and Ito, 1967; Chinya and Ray (1976) and *Antheria mylitta* (Agarwal *et al.*, 1981). Contradictory reports are available in the levels of total lipids during insect metamorphosis. The lipids such as glycerides and phospholipids have been shown to be essential for the oocyte maturation (Rajasekhar, 1993). Agarwal *et al.* (1981) have reported a continuous decline in their levels from the first to fifth instar in *Antheria*. Gupta and Pathak (1984) observed an increase in their levels from the second to fifth instar. Further, it was reported that the fifth instar and mature spinning larvae contain the highest amount of total lipids and that they form the chief source of energy during

prolonged cocoon spinning activity (Kercut and Gilbert, 1985; Inagaki and Yamashita, 1986; Dinakar, 1988).

To understand the rate of energy metabolism under turmeric stress, the author tried to measure two key enzymes namely ATPase and Cytochrome-c oxidase in the tissues and haemolymph of the *B. mori* larvae. The turmeric fed 4th and 5th instar *Bombyx mori* larval intestine, silk gland, haemolymph and muscle showed an increase in their total ATPase activity (Table-10) over the control. Increases in tissue cytochrome-c-oxidase under turmeric stress (Table -11) reflect increase in oxidative metabolism ultimately leading to the generation of more ATP to meet the energy demands of *B. mori* tissues under turmeric stress.

The major input in the manufacturing of raw silk in cocoon that plays an important role in the production and quality of raw silk. Cocoon lots contain varying proportions of defective cocoons, unreelable cocoons, the percentage of which need to be assessed accurately. The quality of cocoons and its quality should be ascertained for achieving optimum productivity and raw silk quality for an established process line.

The overall study confirms that turmeric sprayed mulberry leaf fed silkworm larvae (over a period of 48 hours) enhance the larval synthetic activities followed by improved metabolic functions. In addition, to assess the yield and quality of cocoons and silk from turmeric fed *B. mori* larvae, the author attempted to determine certain of the quantitative parameters like cocoon weight, shell weight, shell percentage, filament length and denier.

Interestingly, all the quantitative parameters excepting the denier of silk from turmeric fed larvae showed increasing trends over the control group

(table 12 and 13) and the trends were observed more for cocoons and silk from 1% turmeric fed 4th and 5th instar *B. mori* larvae. The decreased denier (table 12 and 13) in the silk from turmeric fed cocoons indicative of good quality of silk. The overall experimental data indicate that turmeric fed *B. mori* larvae enhance cocoon yield and the silk will be of quality type and this situation is exerted more by 1% turmeric and less for 10% and 100%. The disease-free state of larvae and cocoons throughout the study might be due to beneficial role of curcumin in boosting the immune system. (Agarwal *et al.*, 2006, Jagetia *et al.*, 2007). This is a much

interesting and welcoming factor to explore further.

CONCLUSION

Based on the overall study, it is reported that turmeric treatment gears the overall protein, free amino acid and lipid metabolisms of the *Bombyx mori* larvae and the turmeric at concentrations tested, enhanced the biomass of the silkworm and this results in improving the cocoon yield. Of all the turmeric concentrations tested, 1% turmeric appeared to be more beneficial in improving the larval biomass and cocoon yield. Interestingly, throughout study *B. mori* larvae and cocoons were found to be disease free.

Table 1: Turmeric induced changes in the levels of total proteins in silkworm, *B. mori* larval tissues and haemolymph (Values expressed as mg / gm wet wt. of tissue or 1 ml of haemolymph)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	65.26	75.52	72.51	66.01	85.21	103.36	98.23	86.49
SD	± 1.02	± 0.927	± 1.36	± 0.825	± 0.793	± 1.72	± 1.44	± 0.653
PC		15.72	11.10	1.14 ^{NS}		20.12	15.27	1.50 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	
Silk gland								
Mean	97.37	118.08	115.25	98.30	115.53	153.61	146.43	116.60
SD	± 2.01	± 2.16	± 1.03	± 1.07	± 2.62	± 1.05	± 2.06	± 1.84
PC		21.6	18.36	0.95 ^{NS}		32.96	26.9	1.01 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	
Haemolymph								
Mean	49.52	56.82	52.25	50.01	62.44	73.07	72.07	63.50
SD	± 0.925	± 0.956	± 0.836	± 0.265	± 1.33	± 0.655	± 0.655	± 0.941
PC		14.74	5.51	0.96 ^{NS}		18.30	15.42	1.69 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	
Muscle								
Mean	71.44	85.00	79.56	72.32	85.34	102.00	98.91	86.53
SD	± 1.08	± 0.944	± 1.76	± 1.72	± 0.942	± 1.30	± 0.722	± 0.723
PC		18.98	11.36	1.23 ^{NS}		19.52	15.90	0.86 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	

Table 2: Turmeric induced changes in the Free Amino Acids (FAA) content in *B. mori* larval tissues and haemolymph (Values expressed mg / gm wet wt. of tissue or 1 ml of haemolymph)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	180.65	210.24	194.32	181.30	210.00	125.50	232.00	212.68
SD	± 1.62	± 2.32	± 1.981	± 0.856	± 1.82	± 1.96	± 2.10	± 1.98
PC		16.37	7.56	0.58 ^{NS}		19.04	10.47	0.95 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	
Silk gland								
Mean	245.00	272.72	267.52	246.12	260.00	300.00	291.12	262.54
SD	± 2.00	± 2.981	± 2.56	± 3.01	± 2.20	± 2.96	± 3.00	± 2.13
PC		11.31	9.19	0.46 ^{NS}		15.38	11.96	0.68 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	
Haemolymph								
Mean	141.22	162.41	158.65	142.00	153.92	182.19	174.36	156.12
SD	± 1.580	± 2.892	± 3.760	± 1.87	± 2.00	± 1.962	± 1.78	± 1.02
PC		15.00	12.34	0.55 ^{NS}		18.36	13.27	1.42 ^{NS}
t		p<0.001	P<0.001			p<0.001	p<0.001	
Muscle								
Mean	240.80	258.19	249.11	242.23	268.41	295.52	286.15	270.85
SD	± 1.74	± 2.05	± 2.99	± 2.86	± 1.789	± 3.00	± 2.985	± 1.25
PC		7.22	3.45	0.59 ^{NS}		10.10	6.60	2.45 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	

Table 3: Turmeric induced changes in protease activity levels in *B.mori* larval tissues and haemolymph (Values expressed as moles of tyrosine equivalent /mg protein/ hour or 1 ml of haemolymph)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	0.493	0.685	0.584	0.514	0.524	0.700	0.625	0.539
SD	± 0.065	± 0.059	± 0.062	± 0.065	± 0.055	± 0.078	± 0.062	± 0.051
PC		38.94	18.45 ^{NS}	4.25 ^{NS}		33.58	19.27	2.86 ^{NS}
t		p<0.001				p<0.001	p<0.05	
Silk gland								
Mean	0.855	1.05	0.950	0.870	1.42	1.80	1.52	1.44
SD	± 0.092	± 0.044	± 0.084	± 0.509	± 0.023	± 0.051	± 0.045	± 0.023
PC		22.80	5.26	1.75 ^{NS}		26.76	7.04	1.40 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	
Haemolymph								
Mean	0.462	0.552	0.526	0.486	0.637	0.792	0.700	0.642
SD	± 0.073	± 0.048	± 0.035	± 0.042	± 0.045	± 0.089	± 0.075	± 0.056
PC		19.48	8.22	0.042 ^{NS}		24.33	9.89	0.78 ^{NS}
t		p<0.01	p<0.01			p<0.001	p<0.05	

Muscle								
Mean	0.910	1.05	1.02	0.926	1.59	1.92	1.68	1.62
SD	±0.0341	± 0.024	± 0.056	± 0.094	± 0.055	± 0.094	± 0.071	± 0.082
PC		15.38	12.08	1.75 ^{NS}		20.75	5.66 ^{NS}	1.88 ^{NS}
t		p<0.001	p<0.01			p<0.001		

Table 4: Turmeric induced changes in the Aspartate amino transferase (AAT) activity levels in *B. mori* larval tissues and haemolymph (Values expressed as moles of pyruvate formed/ mg protein/ hr or 1 ml of haemolymph)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	0.635	0.780	0.725	0.645	0.882	1.20	0.998	0.892
SD	±0.48	± 0.059	± 0.65	±0.042	±0.0624	± 0.056	± 0.093	± 0.074
PC		22.83	14.17	1.57 ^{NS}		36.05	8.84	1.13 ^{NS}
t		p<0.001	p<0.01			p<0.001	p<0.01	
Silk gland								
Mean	1.18	1.43	1.32	1.25	1.45	2.72	2.35	1.50
SD	± 0.074	± 0.096	± 0.054	± 0.083	± 0.054	± 0.721	± 0.647	± 0.524
PC		21.18	12.00	5.93 ^{NS}		24.13	19.31	3.44 ^{NS}
t		p<0.05	p<0.01			p<0.001	p<0.01	
Haemolymph								
Mean	0.425	0.500	0.385	0.432	0.406	0.522	0.451	0.420
SD	± 0.044	± 0.421	± 0.079	± 0.036	± 0.0824	± 0.0472	± 0.0709	± 0.364
PC	19.04	p<0.001	7.84	1.64		28.57	11.08	3.44
t			p<0.001	p<0.05		p<0.001	p<0.05	p<0.05
Muscle								
Mean	0.593	0.906	0.749	0.625	0.631	1.17	0.842	0.706
SD	± 0.063	± 0.507	± 0.093	± 0.044	± 0.327	± 0.763	± 0.454	± 0.0847
PC		52.78	26.30	5.39		85.41	33.43	11.88
t		p<0.001	p<0.001	p<0.001		p<0.001	p<0.05	p<0.05

Table 5: Turmeric induced changes in Alanine aminotransferase (ALAT) activity levels in *B. mori* (Values expressed as mole of pyruvate formed / mg protein/ hr or 1 ml of haemolymph)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	1.01	1.19	1.09	1.03	1.09	1.49	1.21	1.13
SD	± 0.041	± 0.031	± 0.060	± 0.044	± 0.061	± 0.050	± 0.052	± 0.0966
PC		17.8	7.92	1.98		36.69	11.00	3.66 ^{NS}
t		p<0.001	p<0.001	P<0.05		p<0.001	p<0.001	
Silk gland								
Mean	1.30	1.72	1.61	1.35	1.45	2.07	1.86	1.49
SD	± 0.050	± 0.067	± 0.040	± 0.036	± 0.060	± 0.83	± 0.0513	± 0.070
PC		2.30	23.84	3.84 ^{NS}		42.75	28.27	2.75 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	

Haemolymph								
Mean	0.524	0.635	0.582	0.540	0.637	0.885	0.820	0.684
SD	±0.086	± 0.037	± 0.061	±0.082	±0.086	± 0.090	± 0.067	± 0.042
PC		21.18	11.06	3.05 ^{NS}		38.93	28.72	7.37 ^{NS}
t		p<0.001	p<0.001			p<0.001	P<0.001	
Muscle								
Mean	0.605	0.740	0.700	0.617	0.724	0.952	0.833	0.741
SD	± 0.074	± 0.042	± 0.091	±0.050	±0.093	±0.061	± 0.052	± 0.075
PC		22.31	15.70	1.98 ^{NS}		31.41	15.05	2.34 ^{NS}
t		p<0.001	p<0.05			p<0.001	p<0.01	

Table 6: Turmeric induced changes in Glutamate Dehydrogenase (GDH) activity levels in *B. mori* larval tissues and haemolymph (Values expressed as mole of formazan formed /mg protein/ hr or 1 ml of haemolymph)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	0.75	0.96	0.91	0.77	0.91	1.41	1.28	0.95
SD	±0.062	±0.051	± 0.036	±0.046	±0.073	± 0.53	± 0.061	± 0.038
PC		28.00	21.33	2.66 ^{NS}		64.94	40.65	4.39 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	
Silk gland								
Mean	0.64	0.78	0.75	0.68	0.72	0.99	0.92	0.76
SD	±0.044	± 0.032	± 0.066	± 0.051	±0.044	± 0.041	± 0.034	± 0.044
PC		21.87	17.18	6.25 ^{NS}		37.50	27.77	5.55 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	
Haemolymph								
Mean	0.32	0.65	0.44	0.41	0.35	0.78	0.63	0.39
SD	±0.065	± 0.031	± 0.055	± 0.026	±0.025	± 0.041	± 0.035	± 0.056
PC		103.12	37.50	28.12 ^{NS}		122.89	80.00	11.42 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	
Muscle								
Mean	0.55	0.75	0.62	0.59	0.61	0.87	0.75	0.64
SD	± 0.067	± 0.042	± 0.054	± 0.044	±0.035	±0.065	± 0.064	± 0.041
PC		36.36	12.72	7.27 ^{NS}		42.62	22.95	4.91 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	

Each value is the mean ± SD of 10 samples

SD : Standard deviation, PC : Percent change over the control * P< 0.001; NS: Non Significant

Table 7: Turmeric induced changes in levels of total lipids in *B. mori* larval tissues and haemolymph (Values expressed as mg / gm wet wt. of tissue or 1 ml of haemolymph)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	120.05	105.76	112.14	110.66	137.08	102.31	109.26	120.49
SD	±3.22	± 0.345	±1.75	±2.44	± 0.590	± 1.32	±0.520	± 0.812
PC		-11.90	-6.58	-7.82		-25.36	-20.29	-12.10
t		p<0.001	p<0.001	p<0.001		p<0.001	p<0.001	p<0.001
Silk gland								
Mean	142.00	121.21	127.50	125.41	160.00	141.29	146.96	152.72
SD	±2.620	± 1.46	± 2.22	± 0.0590	±0.692	± 3.10	± 2.25	± 2.00
PC		-14.64	-10.21	-11.68		-11.69	-8.15	4.55
t		p<0.001	p<0.001	p<0.001		p<0.001	p<0.001	p<0.001
Haemolymph								
Mean	70.33	55.08	62.21	66.23	82.01	60.52	71.03	74.47
SD	±1.70	± 2.00	± 1.82	± 1.92	± 1.83	± 2.12	± 1.57	± 0.840
PC		-21.68	-11.54	-5.82		-26.20	-13.38	-9.19
t		p<0.001	p<0.001	p<0.001		p<0.001	p<0.001	p<0.001
Muscle								
Mean	99.00	72.33	81.44	84.07	103.94	85.30	96.62	99.73
SD	± 1.82	± 0.569	± 3.50	± 1.82	±0.850	± 0.940	± 0.984	± 1.42
PC		-26.93	-17.73	15.08		-17.93	-7.64	-4.0
t		p<0.001	p<0.001	p<0.001		p<0.001	p<0.001	p<0.0015

Table 8: Turmeric induced changes in the Free Fatty Acids (FFA) content in the silkworm (Values expressed as mg / gm wet wt. of tissue or 1 ml of haemolymph)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	158.64	175.79	172.03	165.44	175.62	199.04	192.04	179.22
SD	±2.26	± 3.74	± 2.609	±1.24	±2.967	± 2.24	± 2.02	± 2.98
PC		10.08	8.44	4.31 ^{NS}		13.33	9.34	2.04
t		p<0.001	p<0.001			p<0.001	p<0.001	p<0.001
Silk gland								
Mean	290.52	316.84	305.66	301.14	292.07	325.11	306.73	301.16
SD	±2.10	± 4.08	± 2.98	± 1.82	± 2.05	± 3.00	± 1.89	± 0.968
PC		8.71	5.21	2.66		11.31	5.02	3.11
t		p<0.001	p<0.001	p<0.001		p<0.001	p<0.001	p<0.001
Haemolymph								
Mean	270.29	290.26	285.50	274.54	278.31	308.36	291.26	280.49
SD	±3.02	± 2.20	± 3.752	± 2.886	± 3.74	± 2.66	± 3.02	± 1.87
PC		7.38	5.64	1.48		10.79	4.69	0.782 ^{NS}
t		p<0.001	p<0.001	p<0.001		p<0.001	p<0.001	
Muscle								
Mean	210.43	230.19	224.72	217.63	232.22	261.54	246.54	239.72
SD	± 9.98	± 0.844	± 1.82	± 0.982	± 4.10	± 1.92	± 1.980	± 2.16
PC		9.39	6.79	3.41		12.63	6.17	3.23
t		p<0.001	p<0.001	p<0.001		p<0.001	p<0.001	p<0.001

Table 9: Turmeric induced changes in the Lipase activity levels in the silkworm (Values expressed as moles of paranitrophenol formed / mg protein/ hr or 1 ml of haemolymph)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	0.046	0.054	0.0504	0.049	0.052	0.068	0.065	0.059
SD	±0.032	± 0.001	± 0.003	±0.008	±0.0050	± 0.005	±0.0090	±0.0070
PC		17.39	8.69	6.52 ^{NS}		30.76	25.00	13.46
t		p<0.001	p<0.001			p<0.001	p<0.001	p<0.05
Silk gland								
Mean	0.032	0.041	0.035	0.034	0.039	0.054	0.047	0.043
SD	±0.006	± 0.007	± 0.005	± 0.004	± 0.0050	± 0.008	± 0.006	± 0.003
PC		28.12	9.37	6.25 ^{NS}		38.46	20.15	10.25 ^{NS}
t		p<0.01	p<0.05			p<0.001	p<0.05	
Haemolymph								
Mean	0.022	0.028	0.025	0.023	0.026	0.038	0.032	0.029
SD	±0.026	±0.0080	± 0.005	± 0.006	± 0.004	± 0.007	± 0.006	± 0.005
PC		27.27	13.63	4.54		46.15	23.07 ^{NS}	11.53 ^{NS}
t		p<0.001	p<0.001	p<0.001		p<0.001		
Muscle								
Mean	0.031	0.040	0.036	0.042	0.037	0.060	0.044	0.041
SD	± 0.004	± 0.023	± 0.006	± 0.007	± 0.009	± 0.005	± 0.003	± 0.006
PC		29.03	16.12	3.22		35.13	18.19 ^{NS}	10.81 ^{NS}
t		p<0.001	p<0.001	p<0.001		p<0.001		

Table 10: Effect of Turmeric on Total ATPase activity levels in the silkworm (Values expressed as moles inorganic phosphorus (Pi) formed/ mg protein /hr)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	8.16	14.00	12.07	10.62	10.07	15.32	12.15	10.49
SD	± 0.309	± 0.725	± 1.62	± 0.508	± 0.844	± 2.39	± 0.882	± 1.34
PC		71.56	47.91	30.14 ^{NS}		52.13	20.65	4.17 ^{NS}
t		P<0.001	p<0.001			p<0.001	p<0.05	
Silk gland								
Mean	11.56	15.06	12.1	12.00	143.84	16.35	15.10	14.14
SD	± 0.961	± 2.33	± 884	± 1.77	± 0.901	± 2.66	± 2.31	± 2.08
PC		30.27	10.81 ^{NS}	3.80 ^{NS}		10.17	1.75 ^{NS}	4.71 ^{NS}
t		p<0.001				p<0.001		
Haemolymph								
Mean	9.42	11.44	11.00	9.65	12.49	15.67	14.71	13.22
SD	±0.463	± 1.22	± 2.05	± 0.658	± 1.25	± 1.08	± 1.67	± 0.862
PC		23.99	16.77	2.44 ^{NS}		25.46	17.77	5.84 ^{NS}
t		p<0.001	p<0.001			P<0.01	P<0.01	
Muscle								
Mean	7.41	8.17	8.00	7.33	8.36	12.50	10.33	9.72
SD	±0.425	± 0.440	± 0.509	± 0.438	± 0.640	± 0.917	± 0.746	± 1.77
PC		23.75	7.96 ^{NS}	1.07 ^{NS}		49.52	23.56	16.26 ^{NS}
t		p<0.001				P<0.001	p<0.01	

Table 11: Turmeric induced changes in Cytochrome- c oxidase activity levels in the silkworm (Values expressed moles of formazan formed/ m protein/hr /1of haemolymph)

Name of the tissue	4 th Instar				5 th Instar			
	Control	Percentage of the turmeric solution			Control	Percentage of the turmeric solution		
		1%	10%	100%		1%	10%	100%
Intestine								
Mean	1.92	2.63	2.01	1.99	01.94	2.86	2.04	2.14
SD	± 0.035	± 0.042	± 0.036	± 0.042	± 0.092	± 0.241	± 0.210	± 0.212
PC		36.97	4.68	3.64		47.42	5.15 ^{NS}	10.30 ^{NS}
t		p<0.05	p<0.05	p<0.05		p<0.001		
Silk gland								
Mean	2.86	4.08	3.16	2.92	2.11	3.75	3.45	2.90
SD	± 0.048	± 0.216	± 0.210	± 0.224	± 0.224	± 0.420	± 0.324	± 0.256
PC		42.65	10.48	2.09		77.72	63.50	37.44
t		p<0.001	P<0.001	p<0.01		p<0.001	p<0.001	p<0.001
Haemolymph								
Mean	0.89	1.44	0.961	0.885	0.85	1.60	1.22	0.920
SD	±0.024	± 0.041	± 0.069	± 0.042	± 0.040	± 0.096	± 0.192	± 0.026
PC		61.79	7.97	0.56 ^{NS}		88.23	43.52	8.23 ^{NS}
t		p<0.001	p<0.05			p<0.001	P<0.001	
Muscle								
Mean	1.62	2.33	2.33	1.67	1.66	2.72	2.00	1.68
SD	±0.038	± 0.1790	± 0.242	± 0.172	± 0.056	± 0.234	± 0.180	± 0.172
PC		43.82	23.45	3.08 ^{NS}		63.85	20.48	1.20 ^{NS}
t		p<0.001	p<0.001			p<0.001	p<0.001	

Each value is the mean ± SD of 10 samples

SD : Standard deviation, PC : Percent change over the control * P< 0.001; NS: Non Significant

Table 12: The quantitative parameters of the cocoons from control and turmeric fed 4th instar silkworm

Name of the quantitative parameter	4 th Instar			
	Control	Percentage of the turmeric solution		
		1%	10%	100%
Cocoon Weight (g)				
Mean	1.562	1.758	1.675	1.521
SD	±0.024	±0.04	±0.094	±0.059
PC		12.54	7.23	-2.62 ^{NS}
t		p<0.001	p<0.001	
Shell Weight (g)				
Mean	0.253	0.315	0.297	0.237
SD	±0.044	±0.012	±0.025	±0.035
PC		24.50	17.39	-6.32 ^{NS}
t		p<0.001	p<0.001	
Shell Percentage (%)				
Mean	16.19	18.52	17.48	15.38
SD	±0.01	±1.05	±1.035	±1.5
PC		14.39	7.96	-5.00 ^{NS}
t		p<0.001	p<0.001	

Filament length(mts)				
Mean	700.00	785.00	745.00	685.00
SD	±2.00	±25.68	±15.36	±18.45
PC		12.14	7.42	-2.14
t		p<0.001	p<0.001	p<0.001
Denier (d)				
Mean	2.39	2.05	2.19	2.32
SD	±0.120	±0.250	±0.040	±0.045
PC		-14.22	-8.36	-2.92 ^{NS}
t		p<0.001	p<0.001	

Table 13: The quantitative parameters of the cocoons from control and turmeric fed 5th instar silkworm, *B. mori*

Name of the quantitative parameter	4 th Instar			
	Control	Percentage of the turmeric solution		
		1%	10%	100%
Cocoon Weight (g)				
Mean	1.682	2.158	2.052	1.560
SD	±0.102	±0.380	±0.45	±0.097
PC		28.29	25.99	-7.25 ^{NS}
t		p<0.001	p<0.01	
Shell Weight (g)				
Mean	0.273	0.382	0.363	0.249
SD	±0.045	±0.054	±0.12	±0.056
PC		39.92	32.96	-8.279 ^{NS}
T		p<0.001	p<0.01	
Shell Percentage (%)				
Mean	16.23	19.28	18.95	15.96
SD	±1.05	±1.07	±1.002	±0.972
PC		18.79	16.75	-1.66 ^{NS}
t		p<0.001	p<0.001	
Filament length (mts)				
Mean	700.00	823.00	760.00	670.00
SD	±25.36	±26.87	±24.45	±23.45
PC		15.49	7.04	-4.28 ^{NS}
t		p<0.001	p<0.001	
Denier (d)				
Mean	2.55	2.35	2.42	2.5
SD	±0.12	±0.25	±0.04	±0.045
PC		-8.20	-5.46	-2.34 ^{NS}
t		p<0.001	p<0.05	

Each value is the mean ± SD of 10 samples

SD : Standard deviation, PC : Percent change over the control * P< 0.001; NS: Non significant

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[MS received 20 May 2012;
MS accepted 11 August 2012]

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