DOI: http://dx.doi.org/10.18782/2582-2845.7862

ISSN: 2582 – 2845 *Ind. J. Pure App. Biosci.* (2019) 7(6), 184-191

Research Article



Biochemical Composition and Commercial Characters of Eri Silkworm Reared on Castor Leaf Fortified with Botanicals

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ABSTRACT

An investigation has been conducted to know the influence of feeding castor (Local pink variety) leaf fortified with botanicals to record the commercial characters and biochemical composition (haemolymph, fat body and silk gland) in White-Plain strain of eri silkworm. The castor leaf was fortified with Tinospora cordifolia, Tribulus terrestris and Withania somnifera at 2, 4 and 6% concentrations and two controls namely Distilled water and Absolute control were included in the investigation for comparison. Results with respect to commercial characters revealed that, matured larval weight, cocoon weight and cocoon yield were significantly higher when the eri larvae fed on castor leaf fortified with Tribulus terrestris at 2% concentration followed by Tinospora cordifolia. Further, shell weight, shell yield, shell ratio and silk productivity were significantly superior with Tinospora cordifolia at 4% followed by Tribulus terrestris at 4%. Pupal weight showed superiority with Tinospora cordifolia at 2% followed by Tribulus terrestris at 2%, while fecundity was significantly more with Tribulus terrestris at 2% followed by Tinospora cordifolia at 6% concentration. In respect of biochemical composition of eri silkworm, total protein content in both haemolymph and fat body were significantly higher in the batch of worms fed on castor leaves fortified with Withania somnifera @ 4%, while with respect to silk gland, it was higher with Tribulus terrestris at 2% concentration. Carbohydrate content in haemolymph, fat body and silk gland tissues were significantly more with Tribulus terrestris at 2% concentration. However, majority of the commercial parameters and biomolecules in eri silkworm were inferior with Absolute control. The results of the study inferred that the fortification of castor leaf with Tribulus terrestris at 2% and Tinospora cordifolia at 4% concentration showed considerable improvement in commercial characters of eri silkworm.

Keywords: Biochemical composition, Botanicals, Commercial characters, Eri silkworm.

INTRODUCTION

Knowledge of silkworm nutrition is of great applied value which involves chemical and physiological activities transforming food into body structures. Insect nutrition primarily deals with biochemical substances that are necessary to activate various metabolic processes resulting in growth and development.

Cite this article: Sannappa, B. (2019). Biochemical Composition and Commercial Characters of Eri Silkworm Reared on Castor Leaf Fortified with Botanicals, *Ind. J. Pure App. Biosci.* 7(6), 184-191. doi: http://dx.doi.org/10.18782/2582-2845.7862

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Legay (1958) stated that silkworm nutrition is a major thrust area of research in sericulture, while Pant (1978) envisaged great scope of utilizing data for proper dietary exploitation of beneficial insects like silkworm and stressed that qualitative and quantitative aspects of yield can be directly increased through proper dietary management.

Economic traits such as larval, cocoon and grainage parameters of silkworm are influenced by the nutritional status of the leaves fed to the worms (Krishnaswami et al., 1971). The quality leaves provided to the worms for feeding has been considered as the prime factor governing the production of good cocoon crop. The leaves with superior quality enhance the chances of reaping good cocoon crop (Ravikumar, 1988). Several researchers opined that the food additives have influenced the qualitative and quantitative aspects of silkworm.

Nutritional quality of castor leaves influences ingesta which are related to the physiology of digestion which subsequently influences the growth and development of eri silkworm and its commercial characters. Enrichment of feeds with adequate amount of probiotics confers health benefits to insects by maintaining or improving their intestinal flora. Nutritional supplements include vitamins, proteins and probiotics when added to larval feed tend to increase nutritional efficiency and economic traits of silkworm (Etebary & Matindoost, 2005).

Plants are the richest source of organic chemicals on the earth and phytochemicals have been reported to influence the life and behavior of different insects (Rajasekaragouda et al., 1997). Importance of research on effect of different fortification agents in silkworm nutrition can be judged from "The principles of co-operating supplements" (House, 1966). The role of plant products having potential growth promoting properties particularly on silkworm is gaining importance in recent years (Murugan et al., 1998). In this direction, an attempt has been made to enrich the castor leaf with botanicals to record their influence on bio-chemical composition and commercial characters of eri silkworm.

MATERIALS AND METHODS Rearing of eri silkworm

Prior to rearing, disinfection of silkworm rearing house was done with 0.5% Asthra solution @ 2.0 l/m². Disease free layings of eri silkworm (Strain: White-Plain) was procured from the Central Sericultural Germplasm Resources Centre, Hosur and were incubated at a temperature of $25\pm1^{\circ}$ C and relative humidity of $75\pm5\%$. The hatched larvae were offered tender local pink castor leaves. Eri silkworm rearing operations were conducted from the day of brushing to spinning as per the procedure outlined by Dayashankar (1982). The average temperature and relative humidity recorded during rearing stood at 28.11°C and 73.21%, respectively.

The eri silkworms were reared in specially designed cages to prevent the mixing of larvae kept treatment and replication-wise as these worms are highly motile in later instars (fourth and fifth). One hundred larvae were used for the experimentation. The ripened eri silkworms were picked up from the rearing trays, transferred to plastic mountages separately and were kept one above the other to avoid migration of larvae between replicates and treatments. The cocoons were harvested on seventh day after mounting.

No.	Common name	Botanical name	Plant part used		
1	Guduchi	Tinospora cordifolia	Leaf		
2	Neggilu	Tribulus terrestris	Leaf		
3	Ashwagandha	Withania somnifera	Leaf		

Botanicals used for the study

T5 = Tribulus terrestris at 4% concentration T6 = Tribulus terrestris at 6% concentration **T7** = *Withania somnifera* at 2% concentration

- **T8** = *Withania somnifera* at 4% concentration
- **T9** = *Withania somnifera* at 6% concentration
- **T10** = Distilled water control
- **T11** = Absolute control

Method of application

Botanical extracts at different concentrations was sprayed on castor leaves and surface dried. The treated leaves were fed to eri silkworms once a day (first feed) during fourth and fifth instar.

Biochemical analysis of eri silkworm

Biochemical constituents in haemolymph, fat body and silk gland samples in 5th day of fifth instar larvae were collected for analysis. The collected tissue samples was preserved in -20°C and used for quantitative estimation using spectrophotometer.

Total protein: Total protein content in haemolymph, fat body and silk gland of eri silkworm were estimated by adopting the procedure of Lowry et al. (1951). The results were showed using standard graph and expressed in mg/ml for haemolymph and mg/g of wet tissue for fat body and silk gland.

Total carbohydrates: Total carbohydrate content in haemolymph, fat body and silk gland of eri silkworm was estimated by adopting the procedure of Anthrone method (Sadasivam & Manickam, 2008). The results was showed using standard graph and expressed in mg/ml for haemolymph and mg/g of wet tissue for fat body and silk gland.

Commercial characters of eri silkworm

The commercial characters of eri silkworm namely matured larval weight, total larval duration, cocoon weight, cocoon yield, shell weight, shell yield, shell ratio, pupal weight

and fecundity were recorded under different treatments.

Statistical analysis of data

The experimental data collected on eri silkworm were analyzed statistically for test of significance using Fisher's method of Analysis of Variance (ANOVA) (Cochran and Cox, 2000) using SPSS statistical package (Ver. 21.0).

RESULTS AND DISCUSSION

Biochemical composition of eri silkworm Total protein

Haemolymph: Eri silkworms fed on local pink castor leaves supplemented with botanicals during fifth instar showed significant difference for protein content in haemolymph. Among the botanicals at different concentrations, the larvae fed on leaf fortified with Withania somnifera @ 4% recorded more protein content (35.41 mg/ml) followed by Tinospora cordifolia @ 6% (35.19 mg/ml), absolute control (33.46 mg/ml), W. somnifera @ 2% (33.38 mg/ml), W. somnifera @ 6% (33.10 mg/ml), distilled control (32.11 mg/ml), water Tribulus terrestris @6% (31.89 mg/ml), T. cordifolia @ 4% (31.58 mg/ml), T. cordifolia @2% (27.58 mg/ml) and T. terrestris @ 4% (25.53 mg/ml). However, protein content in haemolymph was less with T. cordifolia @ 2% (24.50 mg/ml) (Table 1).

Fat body: Significant variation was noticed with respect to total protein content in fat body tissue when the larvae nourished with castor leaf fortified with botanicals. Higher total protein content (24.56 mg/g) was recorded in the batches of larvae fed on castor leaf fortified with W. somnifera @ 4% as compared to T. cordifolia @6% (24.07 mg/g), W. somnifera @2% (24.04 mg/g), T. cordifolia @4% (23.64 mg/g), W. somnifera @6% (23.44 mg/g), absolute control (22.94 mg/g), distilled water control (22.49 mg/g), T. terrestris @ 6% (22.49 mg/g), T. cordifolia @ 2% (20.69 mg/g), T. terrestris @ 4% (20.39 mg/g) and it was lower with T. terrestris @ 2% (20.06 mg/g) (Table 1).

Preparation of botanical extracts

treatment details are furnished below:

Botanical extracts was prepared at different

concentrations using distilled water. The

T1 = *Tinospora cordifolia* at 2% concentration

T2 = *Tinospora cordifolia* at 4% concentration

T3 = *Tinospora cordifolia* at 6% concentration

T4 = *Tribulus terrestris* at 2% concentration

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Silk gland: In silk gland tissue, total protein content did not show significant variation when the larvae reared on local pink castor leaf fortified with botanicals. It ranged between 23.85 mg/g (Absolute control) and 28.73 mg/g (*T. terrestris* @2%) (Table 1).

Total carbohydrates

Haemolymph: Eri silkworms nourished with local pink castor leaves fortified with botanicals during fifth instar showed marked variation in total carbohydrate content in haemolymph. Larvae fed on leaf fortified with T. terrestris @ 2% (19.58 mg/ml) registered more total carbohydrate content as compared to T. cordifolia @ 4% (19.32 mg/ml), distilled water control (19.31 mg/ml), W. somnifera @ 2% (19.10 mg/ml), T. terrestris @ 6% (18.62 mg/ml), T. cordifolia @ 6% (18.60 mg/ml), W. somnifera @ 4% (18.45 mg/ml), W. somnifera @ 6% (18.01 mg/ml), absolute control (17.89 mg/ml), *T. cordifolia* @ 2% (17.49 mg/ml) and it was less with T. terrestris @ 4% (16.41 mg/ml) (Table 1).

Fat body: Total carbohydrate content differ significantly in fat body tissue when the larvae fed on castor leaf fortified with botanicals. Highest total carbohydrate content (15.45 mg/g) was noticed with the batches of larvae reared on castor leaf fortified with *T. terrestris* @ 2% followed by *T. cordifolia* @ 4% (15.27 mg/g), *W. somnifera* @ 2% (14.65 mg/g), distilled water control (14.13 mg/g), *W. somnifera* @ 6% (13.98 mg/g), *W. somnifera* @ 4% (13.97 mg/g), *T. terrestris* @ 6% (13.81 mg/g), *T. cordifolia* @ 6% (13.83 mg/g), absolute control (13.60 mg/g), *T. cordifolia* @ 2% (12.81 mg/g) and it was lowest with *T. terrestris* @ 4% (12.39 mg/g) (Table 1).

Silk gland: In silk gland tissue, total carbohydrate content during fifth instar fifth day found significant when the larvae reared on local pink castor leaf fortified with botanicals. Total carbohydrate content was more (16.02 mg/g) when the larvae nourished on castor leaf fortified with *T. terrestris* @2% as compared to *T. cordifolia* @ 4% (15.85 mg/g), *W. somnifera* @ 2% (15.23 mg/g), distilled water control (14.97 mg/g), *W. somnifera* @ 4% (14.55 mg/g), absolute

control (14.44 mg/g), *T. cordifolia* @ 6% (14.41 mg/g), *T. terrestris* @ 6% (14.38 mg/g), *T. cordifolia* @ 2% (13.38 mg/g) and it was less with *T. terrestris* @ 4% (12.97 mg/g) (Table 1).

The biochemical constituents like protein, carbohydrates, amino acids, nucleic acids, etc. are largely depends on the quality of food and the degree of their utilization in insects (Horie, 1961). Nagata and Kobayashi (1990) revealed that the increases in the protein content of haemolymph and silkgland from the beginning to the end of the fifth instar may be due to active secretion of proteins by other tissues like fat bodies. Horie et al. (1982) observed that the protein content in mid gut increased from first day to third day clearly showed that the digestive activities are high during the early part of fifth instar develop which results in increased accumulation of protein that are then transported to other tissues through the haemolymph for further physiological activities in the larva.

Ravikumar and Sarangi (2004) obtained that the protein content increased gradually from beginning to the end of the fifth day and similar trend was observed in silkgland in all the castor varieties. The protein content in haemolymph and silkgland were 53.69 and 70.21 mg/ml in zebra marked larva followed by light blue coloured larva (51.97and 64.01 mg/ml) and plain larva (49.56 and 61.95 mg/ml), respectively.

Commercial characters of eri silkworm Matured larval weight and total larval duration

Eri silkworm fed on castor leaf fortified with botanicals exhibited marked difference in matured larval weight. Significantly higher values for this parameter was secured when the larvae were fed on castor leaf fortified with *T. terrestris* @2% (8.730 g/larva) as compared to *T. cordifolia* @ 4% (8.724 g), *W. somnifera* @ 4% (8.500 g), *T. terrestris* @ 4% (8.486 g), *W. somnifera* @ 2% (8.442 g), *W. somnifera* @ 6% (8.411 g), *T. cordifolia* @ 2% (8.326 g), distilled water control (8.148 g), *T. cordifolia* @ 6% (7.989 g), *T. terrestris* @ 6% (7.927 g) and it was lower with absolute control (7.905

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g). The eri silkworms fed on castor leaves fortified with botanicals recorded a total larval duration of 22.00 days (Table 2).

Cocoon weight and cocoon yield

Cocoon weight and cocoon yield are the final indicators of the produce from the silkworm rearers point of view. Significantly higher cocoon weight and cocoon yield were recorded when eri worms were fed on castor leaves fortified with T. terrestris @ 2% (3.572g and 85.72 kg/100 layings) as compared to T. cordifolia @ 2% (3.568 g and 85.62 kg), W. somnifera @ 2% (3.346 g and 80.31 kg), T. cordifolia @ 6% (3.248 g and 77.94 kg), W. somnifera @ 6% (3.218 g and 77.23 kg), T. terrestris @ 4% (3.182 g and 76.37 kg), T. cordifolia @ 4% (3.112 g and 74.68 kg), W. somnifera @ 4% (3.076 g and 73.82 kg), distilled water control (2.986 g and 71.67 kg), T. terrestris @ 6% (2.914 g and 69.93 kg) and it was lower with absolute control (2.660 g and 63.84 kg), respectively (Table 2).

Shell weight and shell yield

The cocoons of eri are of open type; hence they are marketed in the form of shells rather than cocoons. Eri silkworms reared on castor leaf fortified with botanicals had significant difference for shell weight and shell yield. Significantly highest shell weight and shell yield were registered when the eri silkworms nourished on castor leaves fortified with T. cordifolia @ 4% (0.591 g and 14.17 kg/100 layings) as compared to T. terrestris @ 4% (0.573 g and 13.75 kg), T. cordifolia @ 6% (0.550 g and 13.21 kg), T. terrestris @ 2% (0.535 g and 12.85 kg), W. somnifera @ 6% (0.508 g and 12.20 kg), T. cordifolia @ 2% (0.507 g and 12.18 kg), W. somnifera @ 4% (0.500 g and 12.00 kg), T. terrestris @ 6% (0.476 g and 11.43 kg), W. somnifera @ 2% (0.459 g and 11.01 kg), distilled water control (0.456 g and 10.96 kg) and it was lower with absolute control (0.428 g and 10.28 kg), respectively (Table 2).

Shell ratio

The ratio of shell to cocoon is important rather than the whole cocoon to obtain higher production of silk. Shell ratio was significantly greater with the larvae fed on castor leaf fortified with T. cordifolia @ 4% (18.91 %) as compared to T. terrestris @ 4% (18.02 %), T. cordifolia @ 6% (17.05 %), T. terrestris @ 6% (16.33 %), W. somnifera @ 4% (16.23 %), absolute control (16.14 %), W. somnifera @ 6% (15.79 %), distilled water control (15.43 %), T. terrestris @2% (15.17 %), T. cordifolia @ 2% (14.25 %) and it was least with W. somnifera @ 6% (13.65 %), respectively (Table 2).

Silk productivity

productivity Silk registered significant variation when the eri larvae fed on castor leaf fortified with some botanicals. More silk productivity was observed in the batches of larvae reared on T. cordifolia @ 4% (8.436 cg/day) followed by T. terrestris @ 4% (8.183 cg), T. cordifolia @ 6% (7.864 cg), T. terrestris @ 2% (7.650%), W. somnifera @ 6% (7.264 cg), T. cordifolia @ 2% (7.250 cg), W. somnifera @ 4% (7.140 cg), T. terrestris @ 6% (6.802 cg), W. somnifera @ 2% (6.554 cg), distilled water control (6.521 cg) and it was less with absolute control (6.116 cg) (Table 2).

Pupal weight

Pupae formed from the eri silkworms fed on leaves fortified with castor botanicals exhibited marked variations with respect to pupal weight. It was significantly higher in the batch of worms nourished with T. cordifolia @ 2% (3.056 g) as compared to T. terrestris @ 2% (3.032 g), W. somnifera @ 2% (2.883 g), W. somnifera @ 6% (2.705 g), T. cordifolia @ 6% (2.693 g), T. terrestris @ 4% (2.605 g), W. somnifera @ 4% (2.572 g), distilled water control (2.526 g), T. cordifolia @ 4% (2.517 g), T. terrestris @ 6% (2.434 g) and it was lower with absolute control (2.228 g) (Table 2).

Fecundity

Fecundity is the final indicator of the grainage parameter for healthy egg production in ericulture. Significantly more fecundity was recorded in the batches of larvae fed on castor leaf fortified with T. terrestris @ 2% (333.3 eggs/laying) followed by T. cordifolia @ 6% (322.6 eggs), T. terrestris @ 4% (316.6 eggs), T. cordifolia @ 4% (315.0 eggs), T. cordifolia @ 2% (300.6 eggs), W. somnifera @ 6%

(286.6 eggs), distilled water control (279.6 eggs), W. somnifera @ 4% (277.0 eggs) and absolute control (274.3 eggs). However, it was less with W. somnifera @ 2% (270.9 eggs) (Table 2).

Sannappa and Manjunath (2012) recorded higher larval weight, cocoon weight, pupal weight, shell weight and shell ratio in Phyllanthus emblica at a concentration of 6 % followed by Allium sativam over absolute control and the rearing performance was inferior when worms fed on castor leaves sprayed with distilled water. The fecundity was higher in Curcuma longa @ 2 and 6 % concentrations followed by Benincasa hispida @ 6 % lower in Cucumis sativus @ 6 %. The hatching percentage was higher in Curcuma longa @ 2 % followed by Phyllanthus emblica @ 6% and it was lower in distilled water.

The fifth instar larval weight exhibited significant result on the first to eight days of administration of plant extracts. The larval weight was significantly maximum when larvae were administered with P. niruri extract (14.80g, 16.77, 18.25, 20.43, 23.42, 25.51, 27.81 and 30.58 g/10 larvae) (Takhlique, 2012).

Castor leaves sprayed with neem oil, pongamia oil and mahua oil (each at 1% and 2%) and fed to eri silkworm from second moult onwards showed that leaves treated with 1 % mahua oil resulted in highest larval weight, lowest mortality of worms and the highest hatching percentage as compared to neem oil and pongamia oil (Mortale et al., 2013).

The experiment was conducted with 50 larvae per treatment with five treatments of botanicals and an untreated control. During fourth instar, least larval weight of 0.38g was recorded in the plain white strain of eri silkworm larvae that were fed with karanj oil (5ml/l) treated leaves followed by the larvae fed with 2 ml/l karanj oil (0.46 g) and 10 ml/l neem oil (0.5g). In brick red strain, the weight of the larva was significantly higher compared to the plain white strain and this strain has shown some resistance to karanj oil treatment. Effective rate of rearing (ERR) varied from 76.84 to 89.9% in plain white strain, whereas in brick red strain, it ranged from 79.88 to 91.46%. Comparatively shorter larval duration was recorded in brick red strain than plain white strain. In plain white strain, significantly higher weight was recorded in the cocoons formed out of worms fed with 3ml/l neem oil (1.89g) and 2 ml/l karanj oil (1.82g) treated leaves. In brick red strain, higher shell weight of 0.64g was recorded with control treatment closely followed by 2 ml/l karanj oil (0.50g) treatment showing its loss of efficacy. The treatmental differences were significant with respect to fecundity and non-significant differences were noticed with egg hatching (Lakshmi Narayanamma et al., 2013).

The botanicals used for supplementation (Alfalfa, Bahola and Jeera) of castor leaves had positive influence on the larval, cocoon and grainage characters in three strains (Blue -Plain, Yellow - Plain and Zebra) of eri silkworm along with distilled water sprayed Thus, the botanicals leaves. can be conveniently used for enhancement of economic characters of eri silkworm (Marak, 2013).

As per Anitha et al (2015), larvae of eri silkworm fed on castor leaves treated with 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% concentrations of Probiotic (Darolac) from third instar onwards had considerably improved the economic parameters like matured larval weight (7.96 g) pupal weight (4.06 g), cocoon weight (4.61g) and shell weight (0.57g), shell ratio (12.17%) and ERR (95%) when compared with control. Among all the 2% concentrations, probiotics (Darolac) showed concentration improvement in parameters such as growth, development as well as commercial qualities of cocoon.

CONCLUSION

From the current investigation, it is pertinent that the White-Plain strain of eri silkworm can be reared on castor leaf fortified with the extracts of botanicals namely T. terrestris @ 2%, T. cordifolia @ 4% and W. somnifera @ 4% concentration to maximize eri cocoon production.

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Sannappa Ind. J. Pure App. Biosci. (2019) 7(6), 184-191 ISSN: 2582 - 2845 Table 1: Efficacy of botanicals at different concentrations on biochemical composition of eri silkworm

		Total protein		Total carbohydrates			
Treatments	Haemolymph	Fat body (mg/g)	Silk gland (mg/g)	Haemolymph	Fat body (mg/g)	Silk gland (mg/g)	
	(mg/ml)			(mg/ml)			
T1 = TC 2%	27.58 ± 0.445	20.69 ± 1.049	27.00 ± 2.225	17.49 ± 0.419	12.81 ± 0.412	13.38 ± 0.412	
T2 = TC 4%	31.58 ± 0.533	23.64 ± 0.250	27.17 ± 2.547	19.32 ± 0.049	15.27 ± 0.284	15.85 ± 0.284	
T3 = TC 6%	35.19 ± 0.839	24.07 ± 1.077	25.58 ± 1.955	18.60 ± 0.579	13.83 ± 0.321	14.41 ± 0.321	
T4 = TT 2%	24.50 ± 1.327	20.06 ± 0.462	28.73 ± 2.040	19.58 ± 1.252	15.45 ± 0.877	16.02 ± 0.877	
T5 = TT 4%	25.53 ± 0.604	20.39 ± 0.832	26.84 ± 1.022	16.41 ± 0.466	12.39 ± 0.034	12.97 ± 0.034	
T6 = TT 6%	31.89 ± 1.270	22.49 ± 1.230	26.80 ± 2.097	18.62 ± 0.316	13.81 ± 0.666	14.38 ± 0.666	
T7 = WS 2%	33.38 ± 0.815	24.04 ± 0.615	26.91 ± 0.795	19.10 ± 0.223	14.65 ± 0.333	15.23 ± 0.333	
T8 = WS 4%	35.41 ± 3.037	24.56 ± 1.435	25.78 ± 1.980	18.45 ± 0.231	13.97 ± 0.188	14.55 ± 0.188	
T9 = WS 6%	33.10 ± 1.618	23.44 ± 0.330	26.54 ± 0.996	18.01 ± 0.209	13.98 ± 0.606	14.82 ± 0.606	
T10 = DWC	32.11 ± 1.517	22.49 ± 0.653	25.09 ± 0.799	19.31 ± 0.020	14.13 ± 0.263	14.97 ± 0.263	
T11 = AC	33.46 ± 1.228	22.94 ± 0.604	23.85 ± 0.359	17.89 ± 0.059	13.60 ± 0.333	14.44 ± 0.333	
Mean	31.25 ± 0.717	22.62 ± 0.342	26.39 ± 0.473	18.43 ± 0.199	13.99 ± 0.192	14.64 ± 0.192	
F- Value	7.182**	3.404*	0.573 ^{NS}	3.824*	4.083*	4.090*	

TC: Tinospora cordifolia TT: Tribulus terrestris AC: Absolute control Non-significant

±: Standard error

WS: Withania somnifera DWC: Distilled water control

*: Significant ($p \le 0.05$) **: Highly significant ($p \le 0.01$) NS:

Treatments	Matured larval weight (g)	Cocoon weight (g)	Cocoon yield (kg/100 layings)	Shell weight (g)	Shell yield (kg/100 layings)	Shell ratio (%)	Silk productivity (cg/day)	Pupal weight (g)	Fecundity (eggs / laying)
T1 = TC 2%	8.326±0.015	3.568±0.110	85.62±2.631	0.507±0.027	12.18±0.651	14.25±0.732	7.250±0.388	3.056±0.103	300.6±12.50
T2 = TC 4%	8.724±0.008	3.112±0.124	74.68±2.981	0.591±0.058	14.17±1.397	18.91±1.233	8.436±0.831	2.517±0.079	315.0±14.40
T3 = TC 6%	7.989 ± 0.028	3.248±0.204	77.94±4.895	0.550 ± 0.007	13.21±0.165	17.05±0.821	7.864 ± 0.098	2.693±0.197	322.6±18.08
T4 = TT 2%	8.730±0.026	3.572±0.334	85.72±8.018	0.535±0.017	12.85±0.418	15.17±1.021	7.650±0.249	3.032±0.322	333.3±2.186
T5 = TT 4%	8.486 ± 0.014	3.182±0.094	76.37±2.253	0.573±0.037	13.75±0.898	18.02 ± 1.186	8.183 ± 0.534	2.605 ± 0.095	316.6±11.93
T6 = TT 6%	7.927±0.062	2.914±0.063	69.93±1.500	0.476 ± 0.015	11.43±0.372	16.33±0.201	6.802±0.221	2.434 ± 0.047	321.6±6.245
T7 = WS 2%	8.442±0.015	3.346±0.198	80.31±4.758	0.459±0.044	11.01±1.053	13.65±0.497	6.554±0.627	2.883±0.154	270.9±7.882
T8 = WS 4%	8.500±0.005	3.076±0.086	73.82±2.073	0.500 ± 0.049	12.00±1.185	16.23±1.419	7.140±0.705	2.572±0.073	277.0 ± 4.096
T9 = WS 6%	8.411±0.015	3.218±0.071	77.23±1.706	0.508±0.029	12.20±0.686	15.79±0.763	7.264 ± 0.408	2.705 ± 0.061	286.6±6.928
T10 = DW	8.148±0.023	2.986±0.155	71.67±3.729	0.456±0.020	10.96±0.492	15.43±1.438	6.521±0.293	2.526±0.175	279.6±9.165
T11 = AC	7.905±0.044	2.660±0.028	63.84±0.679	0.428±0.020	10.28±0.479	16.14±0.859	6.116±0.285	2.228±0.041	274.3±3.844
Mean	8.326±0.050	3.171±0.060	76.10±1.442	0.508±0.012	12.18±0.287	16.09±0.358	7.253±0.171	2.659±0.056	299.8±4.594
F- Value	112.2**	2.975*	2.975*	2.371*	2.371*	2.364*	2.371*	2.965*	5.211*

TC: Tinospora cordifolia

AC: Absolute control

TT: Tribulus terrestris

±: Standard error

WS: Withania somnifera

DWC: Distilled water control

*: Significant (*p*≤0.05)

**: Highly significant $(p \le 0.01)$

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Acknowledgment

This paper is published based on the research results of the grant under 'Minor Research Project' of the University of Mysore, Mysuru.

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Ind. J. Pure App. Biosci. (2019) 7(6), 184-191

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