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.

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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±1°C and relative humidity of 75±5%. 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.

**Botanicals used for the study**

<table>
<thead>
<tr>
<th>No.</th>
<th>Common name</th>
<th>Botanical name</th>
<th>Plant part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Guduchi</td>
<td><em>Tinospora cordifolia</em></td>
<td>Leaf</td>
</tr>
<tr>
<td>2</td>
<td>Neggilu</td>
<td><em>Tribulus terrestris</em></td>
<td>Leaf</td>
</tr>
<tr>
<td>3</td>
<td>Ashwagandha</td>
<td><em>Withania somnifera</em></td>
<td>Leaf</td>
</tr>
</tbody>
</table>
Preparation of botanical extracts
Botanical extracts was prepared at different concentrations using distilled water. The treatment details are furnished below:

T1 = Tinospora cordifolia at 2% concentration
T2 = Tinospora cordifolia at 4% concentration
T3 = Tinospora cordifolia at 6% concentration
T4 = Tribulus terrestris at 2% concentration
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 water control (32.11 mg/ml), 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).
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 silk gland 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 silk gland in all the castor varieties. The protein content in haemolymph and silk gland were 53.69 and 70.21 mg/ml in zebra marked larva followed by light blue coloured larva (51.97 and 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 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% (3.572 g 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 77.32 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* @ 2% (2.914 g and 69.93 kg), *T. terrestris* @ 6% (2.883 g and 71.67 kg), *T. terrestris* @ 2% (2.829 g and 70.39 kg), *T. terrestris* @ 6% (2.705 g), *T. terrestris* @ 2% (2.526 g), *T. terrestris* @ 6% (2.434 g) 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 leaves fortified with botanicals had significant variation when the eri larvae were fed on castor leaves fortified with *T. terrestris* @ 2% (300.6 eggs) as compared to *T. cordifolia* @ 2% (315.0 eggs), *W. somnifera* @ 2% (316.6 eggs), *W. somnifera* @ 4% (315.0 eggs), *T. cordifolia* @ 2% (333.3 eggs/laying) followed by *T. terrestris* @ 4% (300.6 eggs), *T. terrestris* @ 4% (322.6 eggs), *T. terrestris* @ 4% (316.6 eggs), *T. terrestris* @ 2% (300.6 eggs), *W. somnifera* @ 6% (13.65 %), respectively (Table 2).

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(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 sativum 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 treatamental 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 leaves. Thus, the botanicals 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 concentrations, 2% probiotics (Darolac) concentration showed 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.
Table 1: Efficacy of botanicals at different concentrations on biochemical composition of eri silkworm

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total protein</th>
<th>Total carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haemolymph (mg/ml)</td>
<td>Fat body (mg/g)</td>
</tr>
<tr>
<td>T1 = TC 2%</td>
<td>27.58 ± 0.445</td>
<td>20.69 ± 1.049</td>
</tr>
<tr>
<td>T2 = TC 4%</td>
<td>31.58 ± 0.533</td>
<td>23.64 ± 0.250</td>
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<tr>
<td>T3 = TC 6%</td>
<td>35.19 ± 0.839</td>
<td>24.07 ± 1.077</td>
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<tr>
<td>T4 = TT 2%</td>
<td>24.50 ± 1.327</td>
<td>20.06 ± 0.462</td>
</tr>
<tr>
<td>T5 = TT 4%</td>
<td>25.53 ± 0.604</td>
<td>20.39 ± 0.832</td>
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<tr>
<td>T6 = TT 6%</td>
<td>31.89 ± 1.270</td>
<td>22.49 ± 1.230</td>
</tr>
<tr>
<td>T7 = WS 2%</td>
<td>33.38 ± 0.815</td>
<td>24.04 ± 0.615</td>
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<tr>
<td>T8 = WS 4%</td>
<td>35.41 ± 3.037</td>
<td>24.56 ± 1.435</td>
</tr>
<tr>
<td>T9 = WS 6%</td>
<td>33.10 ± 1.618</td>
<td>23.44 ± 0.330</td>
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<tr>
<td>T10 = DWC</td>
<td>32.11 ± 1.517</td>
<td>22.49 ± 0.653</td>
</tr>
<tr>
<td>T11 = AC</td>
<td>33.46 ± 1.228</td>
<td>22.94 ± 0.604</td>
</tr>
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</table>

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* 0.573NS 3.824* 4.083* 4.090*

TC: Tinospora cordifolia TT: Tribulus terrestris WS: Withania somnifera DWC: Distilled water control
AC: Absolute control ±: Standard error *: Significant (p≤0.05) **: Highly significant (p≤0.01) NS: Non-significant

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REFERENCES


