Effect of leaf and seed extracts of *T. cucumerina* L on antibacterial activity and economic traits of mulberry silkworm, *Bombyx mori* L

Shyam Sunder Rudroju, Sampath Akula, Sathish Janga, and Sujatha Kuntamalla

**Abstract**

The present work has been undertaken to study the effect of leaf and seed extracts of *Trichosanthes cucumerina* L in the flacherie disease that infect mulberry silkworm larvae. The mulberry leaves fortified with different concentrations of the extract was fed to the worms orally and its influence on IVth instar larvae, silk gland and economic traits were studied. The methanolic extracts showed maximum antibacterial activity. The various concentrations (1:1, 1:2 and 1:3) of methanolic extracts increased the length and weight of silk gland on alternate days in IVth instar. Both extracts of T1 treatment not only showed highest zone of inhibition against all gram positive and gram negative bacteria but also influenced the larval and cocoon characteristics of *Bombyx mori* L. Where T3 concentration showed lowest zone of inhibition against the bacteria except against *S. albus*, while T2 recorded moderately low antibacterial activity and cocoon weight (1.94±0.11g, 2.1±0.12g), pupal weight (1.54±0.08g, 1.60±0.06g), shell weight (0.39±0.01g, 0.45±0.01g), silk percentage of filament (20.31%, 23%) and filament length (8901±17g, 9000±0.12) were recorded over the control of both the plant extract of leaf and seed respectively.

In the present investigations moderate concentration of *T. cucumerina* extract have showed promoting effect on economical traits of silkworm.

**Keywords:** Mulberry silkworm, plant extracts, zone of inhibition, silk gland and economic traits

1. Introduction

Plants are warehouses of phytochemicals which play a vital role for the development of modern drugs with antibacterial, antifungal, antiviral and anticancer properties, etc. Out of the estimated 2, 50,000 flowering plant species, only 5000 have been thoroughly studied according to the Natural Product Alert (NAPRACERT) data base.

Host plant suitability of phytophagous insects involves nutritional and non-nutritional factors of the host. The feeding behavior of silkworm is altered in response to the nutritional requirements and dietary composition [15] the plant allelochemicals influence the host selection, feeding behavior and population dynamics [13]. Botanicals have immense ability to influence the metabolic activities of insects [9]. Hence, in the past, attempts were made to fortify mulberry leaves with botanical extracts so as to improve the quality of mulberry leaf and feeding efficiency of silkworm and subsequently the silk production [16,11].

In the present investigation, the effect of methanolic extracts of *T. cucumerina* on larval growth and commercial parameters have been examined in the hope of finding that this extract doesn’t have any negative effect on the larvae and can be used in improving the economical traits of silkworm. The extract is prepared from *T. cucumerina* which belongs to family Cucurbitaceae which comprised 130 genera and more than 900 species [16]. The genus *Trichosanthes* is the largest genus of this family.

1.1 Vernacular names

**Telugu**  
Adavipotlakaya

**Hindi**  
Chachinda

**Tamil**  
Pudalankaei

**Sanskrit**  
Amritaphala
1.2 Botanical plant description and chemical constituents
It is a wild plant in Southern India and is closely related to T. angulina. It is an annual climber with leaves 2-4 inches in diameter, obtuse and cordate. Flowers are unisexual and white in color, the fruit is a berry with white stripes when green and red when ripe, it tastes bitter and contains wide range of medicinal properties.

The chemical constituents are cucurbitacin B and E, sterol, β-sitosterol and stigmasterol. It is highly constituted with proteins, fat, fibre, vitamin A and B, total phenol (46.8%) and flavonoid (78%) respectively.

2. Material and Methods

2.1 Plant material
The botanical parts of T. cucumerina, collected from Kakatiya University were shade dried, finely powdered and stored in air tight containers for experimental purpose.

2.2 Preparation of the plant extracts
The shade dried leaves and seeds were powdered individually and 25g of each was extracted with 150 ml of methanol (80%) for 24 hrs in Soxhlet equipment and filtered through 0.45µm membrane filter. These filtrates were evaporated in a rotary evaporator (55 °C) under reduced pressure and used as stock. For experimental purpose, fresh extracts of different concentrations of (leaf and seed: water) were prepared on every third day (T1=1:1, T2=1:2, T3=1:3).

2.3 Antibacterial activity
The used bacteria were Bacillus cereus-KUCC23, Bacillus subtilis-KUCC 17, Staphylococcus aureus-MTCC 96 and Staphylococcus MTCC 96.

2.4 Preparation of bacterial nutrient medium
On the surface of sterile agar plates, standardized 1 to 2x10^8cfu/ml 0.5 MC Farland standards were introduced and inoculums were evenly distributed using a sterile glass spreader, using a sterile cork borer 8mm wells were prepared in the plates in which 50µl of extracts of 200mg/ml concentration was introduced and plates were incubated at 37 °C.

2.5 Preparation of test solution for antibacterial activity
A concentration of 250 mg/ml each methanolic extract of leaf and seed of T. cucumerina was prepared in DMSO (which doesn’t influence the growth of microbes). After 24 hrs, the zone of inhibition was measured with a caliper and 10mg/ml of gentamycin was used as standard. The antibacterial activity results were expressed as mean±SD.

2.6 Procedure for treatment of silkworms
Ten DFLs of Bivoltine dihybrid race were purchased from Vijayawada grainage for the study and reared on V1 mulberry variety in the rearing house as recommended [1]. After second moult, the larvae were selected for the treatment and divided into groups, where each group consisted of three replicates with 100 larvae each. The stock solution (100 ml) was diluted into three different concentrations (T1=1:1, T2=1:2, T3=1:3) with help of a atomizer sprayed on required quantity of mulberry. After shade drying, the silkworms were fed with the fortified mulberry leaves. Mulberry leaves sprayed with distilled water served as control. The observations on economic parameters were studied.

2.7 Isolation of silk gland from silkworm larvae
The silkworm larvae were cut opened longitudinally along the mid dorsal line and a pair of silk gland were removed from the late age larvae, washed twice in ice cold insect ringer and kept in separate Eppendorf tubes containing 200µl of 0.01% SDS and later the silk gland was transferred on to the slide to measure its length.

The silk gland was weighed on an electronic balance in chilled state to record the green weight or wet weight of the silk gland, later it was dehydrated for 24 hrs in the hot oven at 100 °C, which gives the dry weight of the silk gland. The difference in dry and weight was taken as moisture content and its percentage.

2.8 Data Analysis
The data of the parameters were statistically analysed [2]. The statistical analysis of the data was carried out using Student’s t-test and the results were considered significant when p>0.05.

3. Results
The effect of methanolic seed and leaf extract of T. cucumerina were studied not only against flacherie disease but also for the enhancement of economic traits in mulberry silkworm.

3.1 Antibacterial activity of methanolic extracts of leaf and seed
The results of the antibacterial activity of methanolic extract of the selected plant are shown in Table-1 and Figs1&2. The inhibition zone diameters by the test isolates against T. cucumerina was high at T1 concentration in comparison to the standard. The highest antibacterial activity of 10.6 ±0.8mm in Bacillus subtilis at T1 concentration of leaf extract and lowest activity of 3.6±0.4mm in B. cereus was measured. The leaf extract of T. cucumerina exhibited highest activity in the range of 10-7mm against B. subtilis and Staphylococcus aureus and lowest activity in B. cereus at T3 concentration.

The leaf extract has shown the highest inhibition zone (10.6mm) against bacteria followed by the seed extract (9.8 mm) at T1 concentration. Leaf and seed extract of this plant showed high inhibitory activity against B. subtilis and S. aureus at all concentrations when compared to all the other tested bacteria.

| Table 1: Effect of leaf and seed extracts of T. cucumerina on microorganisms infected to silk worm |
|----------------|----------------|----------------|----------------|
|                | Leaf Extracts |                | Seed Extracts |
| Concentration  | Bacillus      | Bacillus       | Bacillus       |
| of seed        | subtilis      | cereus         | staphylococcy  |
| extract        | (ZI in       | (ZI in         | aureus         |
|                | mm±SE)*       | mm±SE)*        | (ZI in          |
|                |               |                | mm±SE)*        |
| T1             | 10.6±0.8      | 5.0±1.2        | 2.5±0.5        |
| T2             | 8.1±0.4       | 5.1±0.4        | 3.3±0.3        |
| T3             | 7.0±0.7       | 3.9±0.2        | 2.8±0.4        |
| Gentamycin     | 5.3±0.7       | 3.0±0.4        | 3.8±0.1        |

* Data presented as mean of 3 readings; ZI = Zone of Inhibition; ±SE=Mean±Standard Error
3.2 Effect of leaf and seed extracts on silk gland

The effect of leaf and seed extract on silk gland was studied on 1st, 3rd, 5th and 7th day and the data was presented in Table 2 and Fig. 3. The green weights of silk gland treated with seed extracts increased from 1st to 7th day over the leaf extract treated batches and control. The values obtained with seed extracts and control on the 7th day for the green weight, dry weight and moisture content are 540 mg, 65 mg and 93.2% and 510 mg, 56 mg and 89.4% respectively.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Age of silk worm (days)</th>
<th>Control</th>
<th>Treatment with leaf extracts</th>
<th>Treatment with seed extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Green weight of SG (mg)</td>
<td>Dry weight of SG (mg)</td>
<td>Moisture content (%)</td>
</tr>
<tr>
<td>1</td>
<td>1st</td>
<td>36</td>
<td>6</td>
<td>88.5</td>
</tr>
<tr>
<td>2</td>
<td>3rd</td>
<td>60</td>
<td>7</td>
<td>92.2</td>
</tr>
<tr>
<td>3</td>
<td>5th</td>
<td>205</td>
<td>23</td>
<td>89.5</td>
</tr>
<tr>
<td>4</td>
<td>7th</td>
<td>510</td>
<td>56</td>
<td>89.4</td>
</tr>
</tbody>
</table>

SG = Silkworm gland
Note: Data is mean of three readings
Plate III: Fig a-f: Effect of leaf extracts of *T. cucumerina* on commercial characters of silk worm a) Leaf extracts in a Petri plate b) Silkworms fed with mulberry leaves fortified with leaf extract c) Fed silkworms with T₁, T₂, T₃ & N=control samples respectively d) Length of the silk gland after various treatments (T₁, T₂, T₃ & N=control) e) Cocoons of *Bombyx mori* (T₁, T₂, T₃ & N=control) (Note the difference in size) f) Skeins samples (g) (T₁, T₂, T₃ & N=control)

Plate IV: Fig a-f: Effect of seed extracts of *T. cucumerina* on commercial characters of silk worm a) Seed extracts in a Petri plate b) Silkworms fed with mulberry leaves fortified with seed extract c) Fed silkworms with N=control, T₁, T₂ & T₃ samples d) Length of the silk gland after various treatments (N=control, T₁, T₂, T₃) e) Cocoons of *Bombyx mori* (N=control, T₁, T₂, T₃) (Note the difference in size) f) Skeins samples (g) (N=control, T₁, T₂, & T₃)
3.3 Linear length of the silk gland in the treated worms

The observations showed linear development of the silk gland of the worms fed with leaves fortified with T. cucumerina are presented in Table 3 and shown in Figs3-4. The linear length of silk gland increased after treatment in comparison to control. Maximum silk gland length was observed in seed extract batches when compared to leaf extract batches.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Age of Silkworm (days)</th>
<th>Control</th>
<th>Treatment with leaf extracts</th>
<th>Treatment with seed extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Length of Anterior Region (cm)</td>
<td>Length of Middle Region (cm)</td>
<td>Length of Posterior Region (cm)</td>
</tr>
<tr>
<td>1</td>
<td>1st</td>
<td>2.2±0.06</td>
<td>1.5±0.02</td>
<td>4.3±0.06</td>
</tr>
<tr>
<td>2</td>
<td>2nd</td>
<td>2.4±0.05</td>
<td>2.4±0.02</td>
<td>4.7±0.05</td>
</tr>
<tr>
<td>3</td>
<td>3rd</td>
<td>3.3±0.03</td>
<td>5.1±0.01</td>
<td>5.6±0.01</td>
</tr>
<tr>
<td>4</td>
<td>4th</td>
<td>3.4±0.06</td>
<td>5.3±0.04</td>
<td>8.0±0.01</td>
</tr>
</tbody>
</table>

3.4 Effect of leaf and seed extracts of T. cucumerina on commercial characters of silkworms

The results of the plant extract’s effect on economic traits are presented in Table 4, significant differences existed among the T. cucumerina extracts treated batches when orally fed to the worms on economic traits of silkworm at different concentrations, seed extract of T. cucumerina at T1 concentration recorded maximum cocoon weight (1.94g), pupal weight (1.54 g), shell weight (0.39g), silk ratio of this plant in control of flacherie disease besides the effect of the extract was not due to one main constituents but due to the combination of other chemical compounds present in it [7]. The maximum inhibition zone at T1 concentration recorded maximum cocoon weight (1.94g), pupal weight and shell weight silk ratio percentage and filament length than control. The above plant extracts at T3 concentration recorded low economic parameters than control. It was recorded that the seed extract showed maximum enhancement of all economic characters studied in comparison to leaf treatments and control (Fig. 4).

Table 4: Effect of leaf and seed extracts of T. cucumerina on commercial characters of silkworm

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Leaf Extracts</th>
<th>Seed Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average cocoon weight (gm)±SEa</td>
<td>Average Pupal weight (gm)±SEa</td>
</tr>
<tr>
<td>T1</td>
<td>1.94±0.11</td>
<td>1.54±0.08</td>
</tr>
<tr>
<td>T2</td>
<td>1.83±0.04</td>
<td>1.48±0.06</td>
</tr>
<tr>
<td>T3</td>
<td>1.74±0.02</td>
<td>1.42±0.03</td>
</tr>
<tr>
<td>C</td>
<td>1.63±0.02</td>
<td>1.38±0.03</td>
</tr>
</tbody>
</table>

4. Discussion

Plant extracts at different concentrations showed tendency not only in improving many of the economic characters but also showed antibacterial activity against the bacteria that infected the silkworms. Bioassay studies against silkworm showed no ill effects after feeding with T. cucumerina. Plant extracts fed larvae enhanced the larval and economic parameters of the silkworm also helped in the control of flacherie disease. Similar findings in silkworm by using the extracts of stem bark of O. indicum to enhance commercial characters in silkworm have also been recorded. The leaf and seed extracts have shown enhanced effect on the economic traits like cocoon, shell and pupal weight which are major traits which influence the silk production and quality. These reports clearly show that the response of silkworm in terms of improvement in economic traits varies with compounds used, silkworm races and geographical region. This positive effect of the extract was not due to one main constituents but due to the combination of other chemical compounds present in it. The maximum inhibition zone at T1 concentration with increase in economic parameters provides a base for use of this plant in control of flacherie disease besides the enhancement of economic parameters in silkworms and thereby helping in discovering a new secondary metabolite for the industry.

5. Conclusion

Thus, based on the investigation it can be concluded that these methanolic extracts on growth and production of the mulberry silkworm are likely to throw light on the possibility of using these extracts not only as a prophylactic measure during silkworm rearing but also for enhancing the commercial characters of silkworm.

6. Acknowledgement

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7. Reference

2. Pillai SK, Simha HC. In statistical methods, for biological workers, Ramprasad and Sons, Agra, 1968.