

## Bioassay Studies of Eri Silk Feeding on Treated Castor Leaves on Eri Silk

### ABSTRACT

**Background:** Cercospora leaf spot of castor, caused by *Cercospora ricinella* Sacc. & Berl. is one of the major foliar diseases encountered in the cultivation of castor for ericulture, causing substantial injury to leaves intended as feed for eri silkworms. Deterioration in the quality or quantity of castor leaves primarily hampers the health of eri silkworm, which ultimately affects the economic parameters of the cocoons produced by them. An experiment was conducted to check the effects of feeding on castor leaves treated with treatments found promising for eco-friendly management of Cercospora leaf spot on the growth of eri silkworm.

**Methods:** Eight treatments were taken for the experiment which included a phytoextract of Tulsi (*Ocimum sanctum*) @ 5%, a bio agent (*Trichoderma viride*) @ 5% and a fungicide (Copper oxychloride) @ 0.2 % and their combinations, applied as foliar spray at 15 days interval on castor plants. These treated leaves were fed as first feed to the 3<sup>rd</sup> instar silkworms after maintaining 25-30 days as safe period of 25-30 days.

**Result:** Silkworms fed with castor leaves treated with *T.viride* + Tulsi extract showed the best results in overall health (ERR 91.66%, Fecundity 391, Shell ratio 13.44%) followed by worms fed with leaves treated with Tulsi extract + *T. viride* + COC (ERR 80.55%, Fecundity 370, Shell ratio 12.59%).

**Key words:** Castor, Cercospora leaf spot, *Cercospora ricinella*, eri silkworm, eri silk, phytochemicals

### INTRODUCTION

Castor (*Ricinus communis* L.) is one of the primary host plants of the eri silkworm *Samia ricini* Donovan, a domesticated polyphagous, multivoltine, sericigenous insect producing the eri silk, also called poor man's silk or the religious doctrine silk, Ahimsa silk (Devaiah *et al.*, 1981; Siddiqui *et al.*, 1993). Ericulture is rooted in the life and culture of the people of the northeast. Presently about 35,062 ha of land is covered under eri plantation and about 2.70 lakh families are engaged in eri culture in the N.E. region of India (Ahmed *et al.*, 2014). Of late, it has been non-traditional states of India like Andhra Pradesh, Chhattisgarh, Tamil Nadu, Karnataka, Maharashtra, Gujarat, Madhya Pradesh, Uttaranchal, Uttar Pradesh, Jharkhand, Bihar, West Bengal, Orissa and Sikkim (Sarmah *et al.*, 2012). Eri accounted for 70.34% (6,946 MT) of the total Vanya raw silk production during 2020-21 (Annual Report, CSB, Bangalore, 2021). Diseases are the major limiting factor in castor cultivation. Feeding silkworms with diseased leaves leads to poor larval growth and disease infections, which ultimately reflects in the qualitative and quantitative parameters of the cocoons produced (Ramanamma, 2009). Cercospora leaf spot is a devastating foliar disease in castor plantations, causing substantial injury to leaves intended as feed for eri silkworms (Chattopadhyay, 2000). The disease is caused by the fungal pathogen *Cercospora ricinella* Sacc. & Berl. In Karnataka, it was reported that in severe conditions, infection by Cercospora leaf spot in castor reduced the yield, production and germination up to 30-50% (Dange *et al.*, 2005). Spraying of copper fungicides may help to bring the disease under check. But where cultures of eri silkworm are maintained on castor plants, spraying is not desirable. Due to the limitations of chemical pesticides for managing seed/soil borne pathogens, biological management has become a widely accepted and alternative way for mitigating the problems. Knowledge on the basic ecology of the biocontrol agents, botanicals or chemicals is very necessary in order to include them in biological control or IDM (Integrated Disease Management). Since we are working with the delicate and sensitive silkworms and we will be feeding these castor leaves to them, it becomes very important for us to improve our understanding of the compatibility and residual effect of the management approaches for the utilization of the control measure in a more efficient way. New research strategies should be focused on alternate eco-friendly ways (Uma *et al.*, 2008), as it seems likely that the agents incorporated in any management practice often has multiple effects on plant growth, soil microflora as well as silkworm health besides managing the target organism. In view of the fact that the eco-friendly measures established during my research for management of Cercospora leaf spot has no side-effects on the health of eri silkworm, the present investigation is carried out to check the effects of the treated castor leaves on eri silkworm.

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- Explain the purpose of the experiment, the results, the place and time of implementation
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## MATERIALS AND METHODS

The rearing was conducted in the rearing room, at College of Sericulture, Titabor, Assam Agricultural University, Jorhat, Assam during 2019-2021. The treated castor leaves of the experiment were collected from the experimental plot 75 days after sowing and utilized for feeding the eri silkworms. The silkworm eggs were collected from Eri Seed Grainage, Dimou and the race of the eggs was Titabor race.

The eri silkworm rearing practices suggested by Sarkar (1980) was followed in rearing eri silkworm for the experiment. The rearing was conducted by tray method of rearing.

The ripe worms were handpicked from the rearing trays and transferred to bamboo mountages (Chandrakis) for cocoon spinning. The cocoons were harvested on 9-10<sup>th</sup> day of mounting.

### Treatment details of castor plants

T<sub>1</sub>: Tulsi extract (*Ocimum sanctum*) @ 5% foliar spray

T<sub>2</sub>: *Trichoderma viride* @ 5% foliar spray

T<sub>3</sub>: Copper oxychloride @ 0.2% foliar spray

T<sub>4</sub>: T<sub>1</sub>+ T<sub>2</sub>

T<sub>5</sub>: T<sub>2</sub>+ T<sub>3</sub>

T<sub>6</sub>: T<sub>1</sub> + T<sub>3</sub>

T<sub>7</sub>: T<sub>1</sub> + T<sub>2</sub> + T<sub>3</sub>

T<sub>8</sub>: Control

### Treatment details of rearing

Season: January-February

Treatments: 8

No. of trays: 8 (One per each treatment)

No. of larvae per tray: 36

Total no. of larvae: 288

### Mode of application

The freshly plucked treated castor leaves were weighed replication-wise and washed under tap water, shade dried and fed to the eri silkworm from third instar daily once as first feed.

### Larval Parameters

#### Larval weight (g)

Ten numbers of eri larvae from each treatment were weighed in an electronic balance at the beginning of the instar and just before mounting/spinning.

#### Larval Duration (days)

The total larval duration was recorded in days from the time of hatching of eggs till 50 per cent larval maturity.

#### Effective rate of rearing, ERR (%)

ERR was calculated using the formula:

$$\text{ERR (\%)} = \frac{\text{Number of cocoons harvested}}{\text{Number of worms brushed}} \times 100$$

### Cocoon parameters

#### Cocoon weight (g)

The cocoon weights from each treatment were recorded in an electronic balance.

**Pupal weight (g)**

After obtaining the cocoon weight, they were cut open and pupal weight was recorded from different treatments.

**Shell weight (g)**

After removing the pupae and larval exuviae from the cocoons, the shell weight was recorded.

**Shell ratio (%)**

The shell ratio was calculated by the formula:

$$\text{Shell ratio (\%)} = \frac{\text{Shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

**Grainage parameters****Moth emergence**

Twenty five cocoons for each replication were preserved for the emergence of moths.

The per cent moth emergence as reflected by the different treatments was computed by the formula:

$$\text{Moth emergence (\%)} = \frac{\text{No. of moths emerged}}{\text{No. of cocoons kept for moth emergence}} \times 100$$

The emerged moths were allowed to mate in cocoon cages treatment wise. Males were separated from females after ensuring 3-4 hours of mating.

**Fecundity**

Moths from each treatment were allowed to lay eggs on kharikas (egg laying apparatus for eri) provided with paper at the bottom and kept in the dark condition for egg laying. The eggs laid by individual females were counted and recorded separately.

**Adult longevity**

From each replication, five moths of both sexes were enclosed separately in perforated plastic containers (20 × 18 cm) to record the longevity of adult. The longevity of adults was calculated by taking the time of emergence and the death of the adults.

**Duration of life cycle (days)**

Duration of life cycle was calculated by adding incubation, larval and pupal periods.

**Statistical analysis**

Data were analysed statistically for test of significance using Fisher's method of "Analysis of variance" as outlined by Sundera Raj *et al.* (1972). The level of significance of 'F' test was tested at 5 per cent. The interpretation of the data was done using critical difference (CD) values calculated at P = 0.05 (Shashidhar *et al.*, 2018).

**RESULTS****Larval Parameters**

Significant effects on larval parameters were noticed when castor leaves with different disease management treatments were fed to eri silkworms from 3<sup>rd</sup> to 5<sup>th</sup> instars as first feed. The treatments with organic components showed better and safer results to silkworms compared to treatments containing copper fungicides, post treatment.

**Larval weight (g)**

Ten worms were selected at random and average larval weight was recorded. Silkworms

fed with castor leaves treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The larval weight of silkworms of T<sub>4</sub> was 8.28 g while that of T<sub>7</sub> was 8.24 g. This was followed by silkworms with treatment T<sub>5</sub> (7.53 g), T<sub>6</sub> (7.46 g), T<sub>2</sub> (7.25 g), T<sub>1</sub> (7.12 g) and T<sub>3</sub> (7.03 g). The least larval weight was observed in silkworms in control *i.e.* T<sub>8</sub> (6.85 g).

#### **Larval duration (days)**

Silkworms fed with castor leaves treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The larval duration of silkworms of T<sub>4</sub> was 44 days while that of T<sub>7</sub> was 47 days. This was followed by silkworms with treatment T<sub>5</sub> (48 days), T<sub>6</sub> (49 days), T<sub>2</sub> (51 days), T<sub>1</sub> (53 days) and T<sub>3</sub> (57 days). The least larval duration was observed in silkworms in control *i.e.* T<sub>8</sub> (58 days).

#### **Effective Rate of Rearing, ERR (%)**

Silkworms fed with castor leaves treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The effective rate of rearing of silkworms of T<sub>4</sub> was 91.66% while that of T<sub>7</sub> was 80.55%. This was followed by silkworms with treatment T<sub>5</sub> (77.77%), T<sub>6</sub> (75.00%), T<sub>2</sub> (72.22%), T<sub>1</sub> (69.44%) and T<sub>8</sub> (58.33%). The least ERR % was observed in silkworms in T<sub>3</sub> (52.77 %).

#### **Cocoon Parameters**

##### **Cocoon weight (g)**

Cocoons from silkworms fed with castor leaves treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The weight of cocoons of T<sub>4</sub> was 2.52 g while that of T<sub>7</sub> was 2.46 g. This was followed by cocoons with treatment T<sub>5</sub> (2.47 g), T<sub>6</sub> (2.02 g), T<sub>2</sub> (3.06 g), T<sub>1</sub> (2.57 g) and T<sub>3</sub> (1.83 g). The least weight was observed in cocoons in control *i.e.* T<sub>8</sub> (2.03 g).

##### **Pupal weight (g)**

Pupae of trays treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The weight of pupae of T<sub>4</sub> was 2.18 g while that of T<sub>7</sub> was 2.15 g. This was followed by pupae with treatment T<sub>5</sub> (2.17 g), T<sub>6</sub> (1.78 g), T<sub>2</sub> (2.70 g), T<sub>1</sub> (2.28 g) and T<sub>3</sub> (1.64 g). The least pupal weight was observed in control *i.e.* T<sub>8</sub> (1.85 g).

##### **Shell weight (g)**

Cocoons of trays treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The shell weight of cocoons of T<sub>4</sub> was 0.34 g while that of T<sub>7</sub> was 0.31 g. This was followed by cocoons with treatment T<sub>5</sub> (0.29 g), T<sub>6</sub> (0.23 g), T<sub>2</sub> (0.35 g), T<sub>1</sub> (0.30 g) and T<sub>3</sub> (0.19 g). The least shell weight was observed in cocoons in control *i.e.* T<sub>8</sub> (0.18 g).

##### **Shell ratio (%)**

Cocoons of trays treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The shell ratio percentage of T<sub>4</sub> was 13.44% while that of T<sub>7</sub> was 12.59%. This was followed by treatment T<sub>5</sub> (11.91%), T<sub>6</sub> (11.78%), T<sub>2</sub> (11.61%), T<sub>1</sub> (11.54%) and T<sub>3</sub> (10.19 %). The least shell ratio was observed in control *i.e.* T<sub>8</sub> (9.02%).

#### **Grainage parameters**

##### **Moth Emergence (%)**

Silkworms fed with castor leaves treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The moth emergence of pupae of T<sub>4</sub> was 93.20% while that of T<sub>7</sub> was 90.07%. This was followed by silkworms with

treatment T<sub>5</sub> (87.98%), T<sub>6</sub> (86.67%), T<sub>2</sub> (85.93%), T<sub>1</sub> (83.79%) and T<sub>3</sub> (71.37%). The least moth emergence per cent was observed in pupae in control *i.e.* T<sub>8</sub> (67.31%).

#### **Fecundity**

Silkworms fed with castor leaves treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The fecundity of moths of T<sub>4</sub> was 391 while that of T<sub>7</sub> was 370. This was followed by silkworms with treatment T<sub>5</sub> (322), T<sub>6</sub> (307), T<sub>2</sub> (298), T<sub>1</sub> (273) and T<sub>8</sub> (265). The least fecundity was observed in moths in T<sub>3</sub> (253).

#### **Adult longevity (hours)**

Silkmoths of trays with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The larval duration of silkworms of T<sub>4</sub> was 216 hrs while that of T<sub>7</sub> was 192 hrs. This was followed by silkworms with treatment T<sub>5</sub> (168 hrs), T<sub>6</sub> (144 hrs), T<sub>2</sub> (120 hrs), T<sub>1</sub> (120 hrs) and T<sub>3</sub> (96 hrs). The least larval weight was observed in silkworms in control *i.e.* T<sub>8</sub> (96 hrs).

#### **Duration of life cycle (days)**

Silkworms fed with castor leaves treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC). The duration of life cycle of T<sub>4</sub> was 80 days while that of T<sub>7</sub> was 83 days. This was followed by silkworms with treatment T<sub>5</sub> (84 days), T<sub>6</sub> (85 days), T<sub>2</sub> (87 days), T<sub>1</sub> (89 days) and T<sub>3</sub> (93 days). The least life cycle duration was observed in silkworms in control *i.e.* T<sub>8</sub> (94 days).

#### **DISCUSSIONS**

The treatments with organic components showed better and safer results to silkworms compared to treatments containing copper fungicides, post treatment. Silkworms fed with castor leaves treated with treatment T<sub>4</sub> (*T. viride* + Tulsi extract) showed the best results and was *at par* with treatment T<sub>7</sub> (Tulsi extract + *T. viride* + COC) while those treated with Copper oxychloride were the worst. As reported by Prasad and Jayalakshmi (2012), Vidyadevi and Ramani Bai (2015) and Sujatha *et al.* (2015), 2% of *O. sanctum* extract has been found to be most effective, having growth promoting effect on silkworm, which helps to enhance the commercial qualities of silk and can be used in sericulture for yield improvement. Bioagents *Trichoderma harzianum* and *Trichoderma viride* possessed antagonistic properties against *Beauveria bassiana* (Rashid and Gulzar, 2020) and were found to be safe to silkworms at 15 per cent concentration when leaves fed to them three days after treatment with culture filtrate of bioagents (Bagwan, 2010). The results of the toxicological studies revealed that *Trichoderma viride* had positive effect on both larval weight and its length. Similarly, cocoon, pupal, shell weight and shell ratio also increased. Effective rate of rearing (ERR) of 91.66% was the highest compared to other treatments. Previous work revealed that application of the chemical fungicides to mulberry plants increased caterpillar mortality up to threefold and significantly reduced the size of the cocoons spun by surviving caterpillars, causing silk production losses even 30 days after application of fungicide (Nicodemo, 2018). The copper fungicide did not interfere with the silkworms' food consumption, but it negatively affected mortality rate and weight of cocoons (Nicodemo, 2018). All the economic traits of male and female adults treated with fungicide decreased (Dutta *et al.*, 2003) but this decrease is not significant in cocoon shell weight and hatching percentage (Bizhannia *et al.*, 2005).

#### **CONCLUSIONS**

Based on the experimental results, it is concluded that silkworms fed with castor leaves treated with treatment *T. viride* + Tulsi extract showed the best results and was *at par* with treatment Tulsi extract + *T. viride* + COC. Copper oxychloride and control showed the highest mortality. The residual effects of chemical fungicides always remain as a threat to silkworm mortality and its economic traits. Chemical fungicides should be opted for host plant disease management only in case of exigencies by maintaining a safe period of minimum 30 days.

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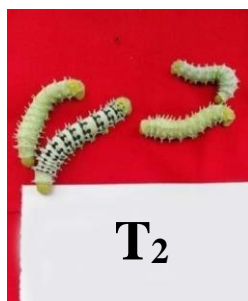
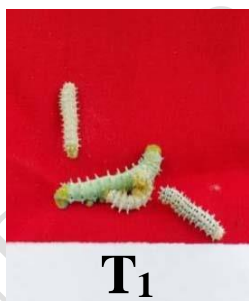
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**T3**

**T4**



Plate 1. Effect of treated castor leaves on larval parameters

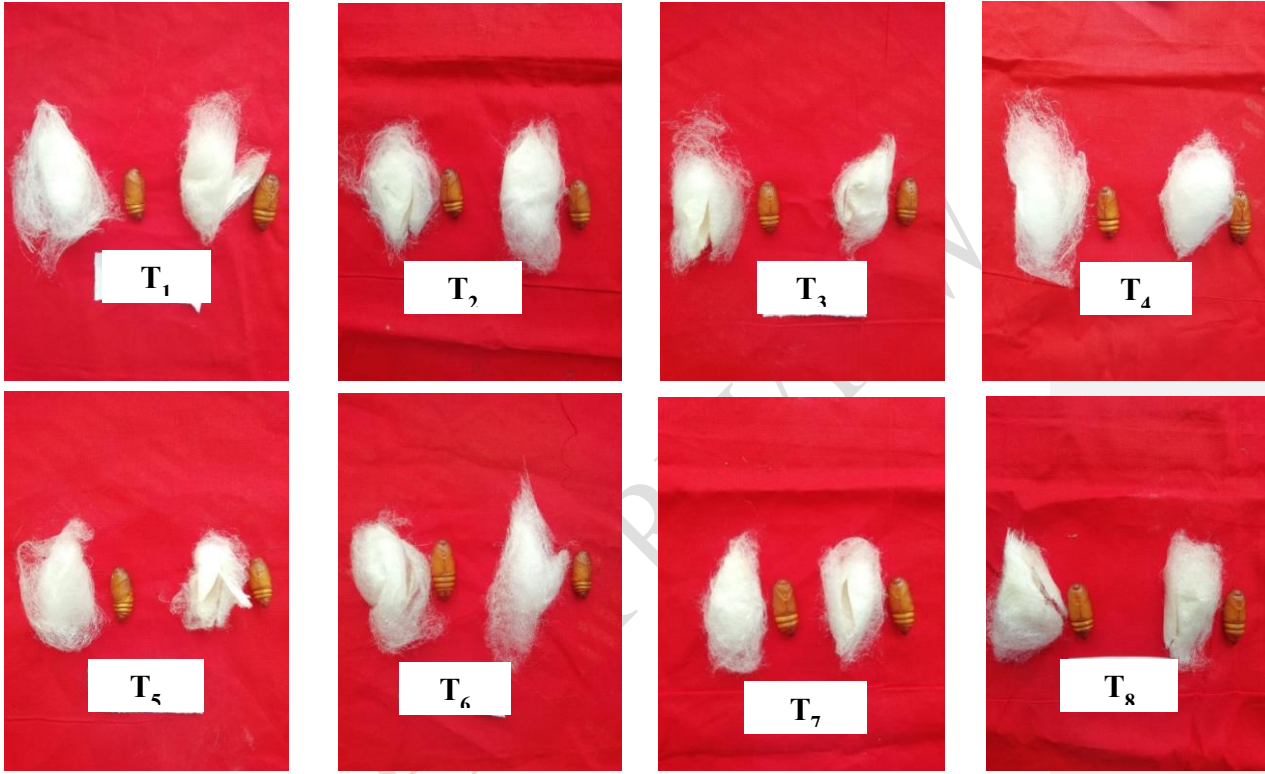
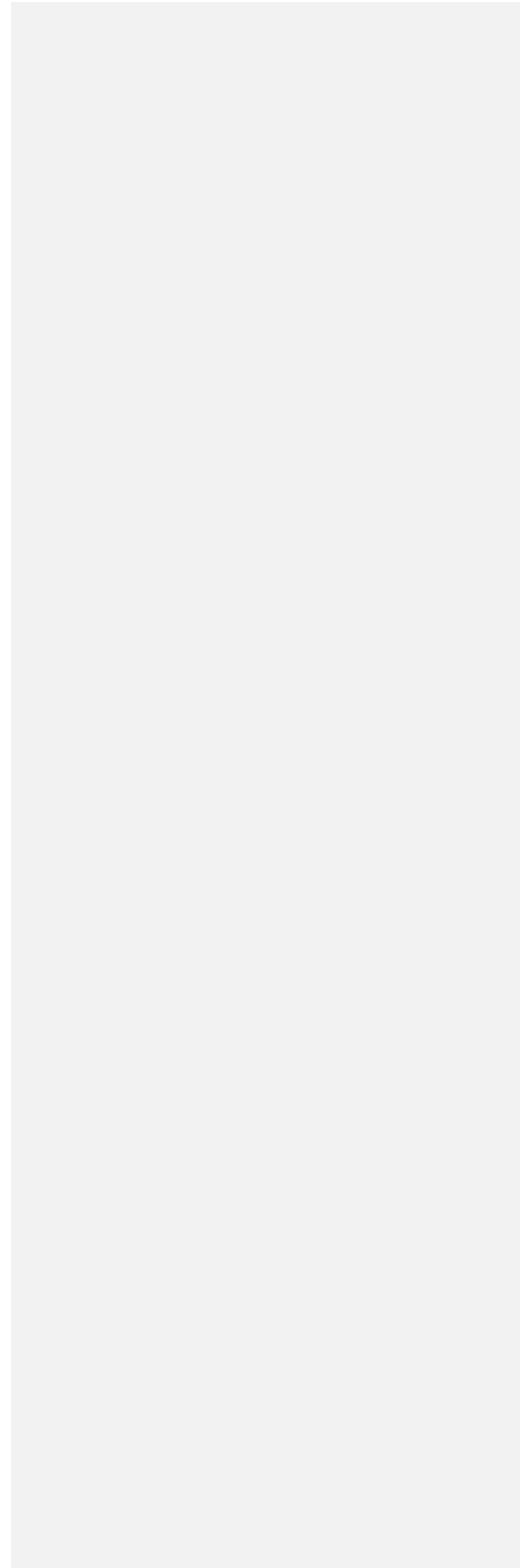


Plate 2. Effect of treated leaves on cocoon parameters of eri silkworm



**Plate 3. Effect of treated leaves on fecundity**

UNDER PEER REVIEW



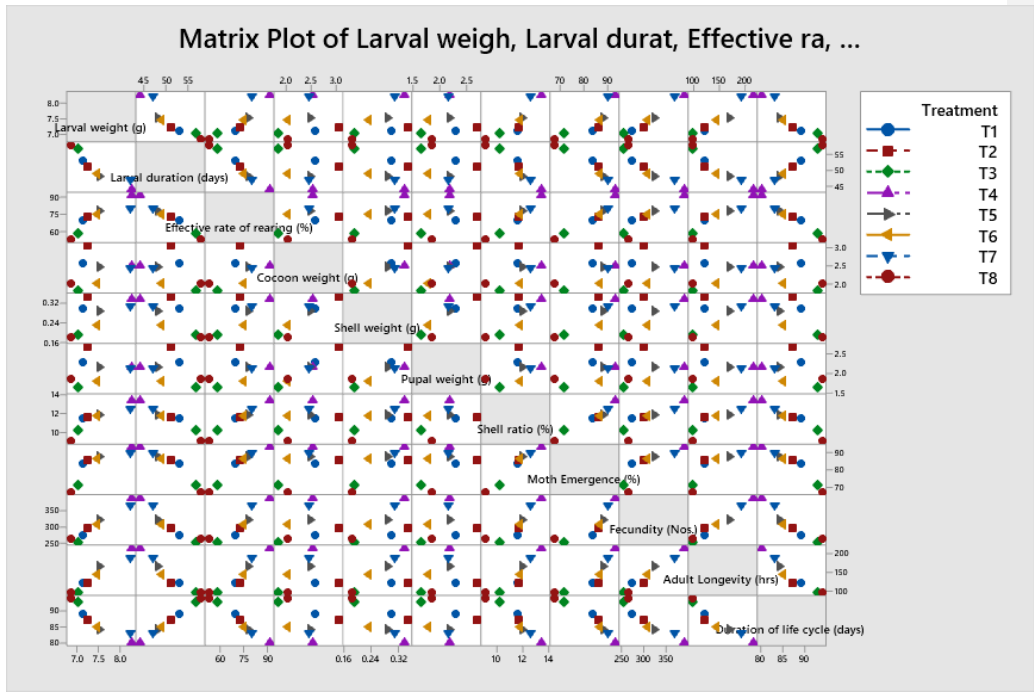


Fig1: Matrix plot of Larval, Cocoon and Grainage parameters of treated eri silkworm (*Samia ricini* Donovan)

UNDER REVIEW

Table 1. Effect of treated castor leaves on larval, pupal and grainage parameters

Treatment	Larval weight (g)	Larval duration (days)	Effective rate of rearing (%)	Cocoon weight (g)	Shell weight (%)	Pupal weight (g)	Shell ratio (%)	Moth Emergence (%)	Fecundity (Nos.)	Adult Longevity (hrs)	Duration of life cycle (days)
T <sub>1</sub>	7.12	53	69.44	2.57	0.30	2.28	11.54	83.74	273	120	89
T <sub>2</sub>	7.25	51	72.22	3.06	0.35	2.70	11.61	85.93	298	120	87
T <sub>3</sub>	7.03	57	58.33	1.83	0.19	1.64	10.19	71.37	253	96	93
T <sub>4</sub>	8.28	44	91.66	2.52	0.34	2.18	13.44	93.20	391	216	80
T <sub>5</sub>	7.53	48	77.77	2.47	0.29	2.17	11.91	87.98	322	168	84
T <sub>6</sub>	7.46	49	75.00	2.02	0.23	1.78	11.78	86.67	307	144	85
T <sub>7</sub>	8.24	47	80.55	2.46	0.31	2.15	12.59	90.07	370	192	83
T <sub>8</sub>	6.85	58	52.77	2.03	0.18	1.85	9.02	67.31	265	96	94
S.E <sub>d</sub> ±	0.02	0.47	0.58	0.01	0.01	0.01	0.11	0.13	2.82	0.07	0.47
C.D. (P=0.05)	0.06	1.41	1.75	0.03	0.02	0.04	0.34	0.40	8.46	0.21	1.41

S.E<sub>d</sub>: Standard Error of Difference; Significance level = (P=0.05)