

Effect of the Essential Oil *Aloe vera* on the Cocoon Traits of Multivoltine Mulberry Silkworm *Bombyx mori* Linn

Priyanka Singh, Surendra Prasad and V.B. Upadhyay

Department of Zoology, D.D.U. Gorakhpur University Gorakhpur. 273009. U.P. India

Abstract: *Bombyx mori* has an economic importance because of the commercial values of its silk. The economic characters of the silk cocoon were reported to improve by the silkworm with mulberry leaves treated with essential oil of *Aloe vera*. The experiments were conducted with *A. vera* oil viz, 0.25, 0.50, 0.75 and 1.0 ml with respect to the treatment of 3rd, 4th and 5th instar *B. mori* larvae. A control set was also arranged for each larval instar. The length of cocoon and volume of cocoon increased with increasing stage of larval treatment and amount of *A. vera* oil up to 0.75 ml, the length of cocoon reached to the maximum level of (3.03±0.72 cm) and volume of cocoon (3.81±0.76 ml) in case of triple treated with 0.75 ml *A. vera* oil. Length of cocoon reached to the minimum level of (2.20±0.77 cm) and volume of cocoon (2.73±0.74 ml) in case of triple treated with 1.0 ml *A. vera* oil. Thus application of *A. vera* oil in sericulture industry may be effective for boost up the growth promoting activity in multivoltine mulberry silkworm, *B. Mori* (L).

Key words: Cocoon Length • Cocoon Volume

INTRODUCTION

The mulberry leaves are the sole source of food for the larval instars of silkworm, *Bombyx mori* L. No doubt, India is the second largest producer of mulberry silk next only to China [1]. The length and Volume of cocoon are the most important factors, which influence the production of cocoon at commercial scale. In order to increase the production of silk, attempts have been made in sericulture to study the effect of temperature [2], relative humidity [3], ecological factors [4], egg magnetization [5,6], cocoon magnetization [7], cocoon refrigeration [8], 20-hydroxyecdysone hormone [9] and phytoecdysteroid hormone [10,11], on the performance of silkworm. In recent years, many attempts have been made to improve the quality and quantity of silk [12] through enhancing the leaves with nutrients, spraying with antibiotics, juvenile hormone, plant products, with JH-mimic principles or using extracts of plants. The plant extracts or phytochemicals could benefit sericulture by improving the silk yield of *B. Mori* and commercial silk production [13]. Human has benefited from the silk, produced by silkworm and subsequently researchers have always been trying to unveil the factors that can be

manipulated to the benefit of the silkworm breeders [14]. Enhancing the leaves with essential oil compounds, are gaining importance because of their wide spectrum of biological action, novel mode of action and eco-friendly nature [15].

A. vera herbal tonic 'Iogen' [16] aloe [17] and Aloe tonic treated mulberry leaves [18] influence the cocoons, pupal and growth parameters of *B. mori*. The quality of the leaves has a profound effect on the superiority of silk produced by *B. mori* L. [12, 19, 20]. *A. Vera* (L) contains over 75 nutrients and 200 active compounds, including vitamins, enzymes, minerals, sugars, lignin, anthraquinones, saponins, salicylic acid and amino acids [21]. Preparation of *Aloe* are, therefore used both topically and as dietary supplement [22]. It is hypothesized that if the larvae of *B. mori* are feed/treated to *A. vera* oil in different amount there may be some beneficial effect on the life cycle of silkworm (*B. mori* L.).

MATERIALS AND METHODS

Seed cocoons: The seed cocoon of multivoltine mulberry silkworm (*B. mori*), a native of West Bengal in India, were obtained from the silkworm grainage. Directorate of

Sericulture, Behraich Uttar Pradesh and were maintained in the plywood trays (23×20×5cm) under the ideal rearing conditions [23] in the silkworm laboratory, Department of Zoology, DDU Gorakhpur university Gorakhpur. The temperature and relative humidity were maintained at $26\pm1^{\circ}\text{C}$ and $80\pm5\%$, respectively till the emergence of moths from the seed cocoons. The moths emerged generally in the morning at around 4 AM.

Copulation: Adult moths have a tendency to pair immediately after emergence and therefore, the female moths required copulating with the male moths were allowed to mate at $26\pm1^{\circ}\text{C}$ and $80\pm5\%$ RH in 12 hour / day dim light condition. After four hours of mating, the paired moths were decoupled manually by holding the female moths between the thumb and middle finger gently and pushing the male away by the forefinger. The male moths were discarded while the female moths were allowed to lay eggs.

Rearing of Larvae: After two consecutive days of hatching, the silkworm larvae were collected with the help of feather of birds and reared to maintain a stock culture in the silkworm laboratory at $26\pm1^{\circ}\text{C}$ and $80\pm5\%$ RH and 12 ± 1 hours light a day. Four feedings of the small pieces of fresh and clean leaves of *Morus alba* were given to the larvae and care was taken that food always remained in excess in the rearing trays. 3rd, 4th and 5th instar larvae were taken for observation.

Experimental Design: To observe the influence of *A. vera* oil on the length of cocoon and volume of cocoon of *B. mori*. The experiments were performed with different doses of *A. vera* oil with respect to the treatment of 3rd, 4th and 5th instar *Bombyx mori* larvae. The larvae of silkworm, *B. mori* (L) were reared laboratory in BOD incubator through the well esteemed method [23]. *A. vera* oil purchased from the Katyani Exports Delhi, India. Four amount of *A. vera* oil viz. 0.25, 0.5, 0.75 and 1.0 ml were uniformly sprayed over mulberry leaf separately by sprayer for 10 minutes before given for feeding to the larvae as 100 g mulberry leaves / 100 larvae. Three set of experiments were designed as single, double and triple treatment of larvae. A control set was also arranged. All the experiments were conducted in the BOD incubator. The experiments were conducted on normal rearing condition i.e. $26\pm1^{\circ}\text{C}$ temperature, $80\pm5\%$ relative humidity and 12 ± 1 hour photoperiod a day.

Single Treatment of Larvae: Single treatment of larvae was performed with the fifth instar larvae. Just before two days of the beginning of larvae spinning. Hundred larvae

were taken out from the BOD incubator and the mulberry leaf treated with 0.25ml amount of *A. vera* oil was given as food further the treated larvae were given normal mulberry leaf for food.

Double Treatment of Larvae: Double treatment of larvae started from the 4th instar larvae. In the first treatment, 100 larvae of fourth instar were treated just before two days of 4th moulting by providing treated mulberry leaf as food with 0.25 ml amount of *A. vera* oil. The treated larvae then transferred in BOD incubator for further rearing and development. Further second treatment for the same larvae was given at the final stage of 5th instar larvae i.e. just before two days of spinning.

Triple Treatment of Larvae: For triple treatment, the 3rd instar larvae just before 3rd moulting were separated from BOD incubator. In the first treatment, 100 larvae of 3rd instar were treated by providing treated mulberry leaf and kept in BOD incubator for rearing. The second treatment of same larvae was done just before two days of 4th moulting i.e. at the final stage of 4th instar larvae and transferred in BOD of spinning. Thus, in the triple treatment 3rd, 4th and 5th instar larvae were treated.

Similar experiments were performed by 0.50, 0.75 and 1.0 ml amount of *A. vera* oil. A control set was always maintained with each set of experiment.

Length of Cocoon: The length of cocoon was taken by cutting the cocoon from the middle in length. The average length of cocoon (three batches of 10 cocoons in each batch) was recorded for each replicate. Three replicates of each experiment were made.

Volume of Cocoon: To observe the volume of cocoon, took healthy cocoons and cut slightly at the top end and removed the pupae. The empty cocoon was filled by water with the help of pipette and the volume of required water was measured in ml. For the average volume of cocoon, 30 cocoons (three batches of 10 cocoons in each batch) were filled with water for each replicate. Three replicates of each experiment were made.

RESULTS

Length of Cocoon: It is clear from the data given in the (Table 1a) that variation in the amount of *Aloe vera* oil and the number of larval treatment influenced the length of cocoon. With the increasing number of larval treatment with 0.25, 0.5, 0.75 ml amount of *A. vera* oil the length of cocoon increased gradually and reached to the maximum level of 3.03 ± 0.72 cm in cases of triple treated larvae with

Table 1a: Effect of essential oil *Aloe vera* on the cocoon length (cm) of *Bombyx mori*.

Stage of treatment (larval instar)	<i>Aloe vera</i> oil applied (ml)					F ₁ -ratio n ₁ = 4
	Control (X ₁)	0.25 (X ₂)	0.50 (X ₃)	0.75 (X ₄)	1.00 (X ₅)	
Single (5 th)	2.22±0.87	2.33±0.81	2.45±0.82	2.54±0.88	2.29±0.85	8.917*
Double (4 th -5 th)	2.22±0.87	2.43±0.88	2.60±0.84	2.75±0.76	2.24±0.86	
Triple (3 rd -5 th)	2.22±0.87	2.55±0.79	3.00±0.92	3.03±0.72	2.20±0.77	
F ₂ -ratio = 3.319**	n ₂ = 2					

*P₁< 0.01

**Non Significant

Each value represents mean±S.E. of three replicates

X₁, X₂, X₃, X₄ and X₅ are the mean values of cocoon length (cm) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectivelyTable 1b: Post-hoc test showing effect of essential oil *Aloe vera* on the cocoon length (cm) of *Bombyx mori*

Mean difference in between groups	Stage of treatment		
	Single	Double	Triple
X ₁ ~ X ₂	0.11	0.21	0.33
X ₁ ~ X ₃	0.23	0.38	*0.78
X ₁ ~ X ₄	0.32	*0.53	*0.81
X ₁ ~ X ₅	0.07	0.02	0.02
X ₂ ~ X ₃	0.12	0.17	*0.45
X ₂ ~ X ₄	0.21	0.32	*0.48
X ₂ ~ X ₅	0.04	0.19	0.35
X ₃ ~ X ₄	0.09	0.15	0.03
X ₃ ~ X ₅	0.16	0.36	*0.80
X ₄ ~ X ₅	0.25	*0.50	*0.83

$$\begin{aligned}
 \text{Honesty significant difference (HSD)} &= q \sqrt{\frac{\text{MS within}}{n}} \\
 &= 5.05 \sqrt{\frac{0.021}{3}} \\
 &= 0.422
 \end{aligned}$$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X₁, X₂, X₃, X₄ and X₅ are mean values of cocoon length (cm) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.

0.75 ml amount of *A. vera* oil. In case of larval treatment with 1.0 ml amount of *A. vera* oil, the length of cocoon decreased in single treated larvae and reached to the minimum level of 2.20±0.77 cm in triple treated larvae with 1.0 ml amount of *A. vera* oil.

Two way ANOVA indicates that the variation in the *A. vera* oil treatment significantly (P₁< 0.01) influenced the length of cocoon (Table 1a). While the Post hoc test indicates significant group difference in the length of cocoon in between control and 0.75 ml, 0.75 and 1.0 ml amount of *A. vera* oil in case of double treatment. In triple treatment significant group difference in the length of cocoon was observed in between control and 0.50 ml, control and 0.75 ml, 0.25 and 0.50 ml, 0.5 and 1.0 ml and 0.75 and 1.0 ml. No group difference was found in case of single treatment (Table 1b, HSD = 0.424).

Volume of cocoon:- The data given in (Table 2a) clearly indicates that variation in the amount of *A. vera* oil and the number of treatment influenced the volume of cocoon. With the increasing number of treatment from one to three times, the volume of cocoon increased in case of 0.25, 0.5, 0.75 ml amount of *A. vera* oil while in case of 1.0 ml amount of *A. vera* oil, the volume of cocoon decreased in single treatment of *Bombyx mori* larvae. The trend of increase in the volume of cocoon with the increasing number of treatment has recorded to be almost of similar in case of 0.25, 0.5, 0.75 ml amount of *A. vera* oil treatment. The maximum volume of cocoon was noticed to be 3.81±0.76 ml in the triple treatment with 0.75 ml amount of *A. vera* oil treatment. The minimum volume of cocoon was recorded 2.73±0.75 ml in case of triple treatment by 1.0 ml amount of *A. vera* oil.

Table 2a: Effect of essential oil *Aloe vera* on the cocoon volume (ml) of *Bombyx mori*

Stage of treatment (larval instar)	<i>Aloe vera</i> oil applied (ml)					F ₁ -ratio n ₁ = 4
	Control (X ₁)	0.25 (X ₂)	0.50 (X ₃)	0.75 (X ₄)	1.00 (X ₅)	
Single (5 th)	3.03±0.78	3.26±0.83	3.42±0.74	3.63±0.77	2.93±0.80	38.053*
Double (4 th -5 th)	3.03±0.78	3.36±0.86	3.62±0.78	3.71±0.82	2.89±0.74	
Triple (3 rd -5 th)	3.03±0.78	3.46±0.75	3.74±0.79	3.81±0.76	2.73±0.75	
F ₂ -ratio = 1.229**	n ₂ = 2					

*P₁< 0.01

**Non Significant

Each value represents mean±S.E. of three replicates

X₁, X₂, X₃, X₄ and X₅ are the mean values of cocoon volume (ml) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively

Table 2b: Post-hoc test showing effect of essential oil *Aloe vera* on the cocoon volume (ml) of *Bombyx mori*

Mean difference in between groups	Stage of treatment		
	Single	Double	Triple
X ₁ ~ X ₂	0.23	*0.33	*0.43
X ₁ ~ X ₃	*0.39	*0.59	*0.71
X ₁ ~ X ₄	*0.60	*0.68	*0.78
X ₁ ~ X ₅	0.10	0.14	*2.70
X ₂ ~ X ₃	0.16	0.26	0.28
X ₂ ~ X ₄	*0.37	*0.35	*0.35
X ₂ ~ X ₅	*0.33	*0.47	*0.73
X ₃ ~ X ₄	0.21	0.09	0.07
X ₃ ~ X ₅	*0.70	*0.73	*1.01
X ₄ ~ X ₅	*0.70	*0.90	*1.08

$$\begin{aligned}
 \text{Honesty significant difference (HSD)} &= \sqrt{q \frac{\text{MS within}}{n}} \\
 &= 5.05 \sqrt{\frac{0.011}{3}} \\
 &= 0.303
 \end{aligned}$$

MS = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X₁, X₂, X₃, X₄ and X₅ are mean values of cocoon volume (ml) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.

Two way ANOVA indicates that the variation in the amount of *A. vera* oil treatment significantly (P₁< 0.01) influenced the volume of cocoon (Table 2a). while the Post hoc test indicates significant group difference in the volume of cocoon in between control and 0.50 ml, control and 0.75 ml, 0.25 and 0.75 ml, 0.25 and 1.0 ml, 0.50 and 1.00 ml and 0.75 and 1.00 ml amount of *A. vera* oil in case of single treatment. In double treatment significant group difference in the volume of cocoon was observed in between control and 0.25 ml, control and 0.50 ml, control and 0.75 ml, 0.25 and 0.75 ml, 0.25 and 1.0 ml, 0.50 and 1.00 ml, 0.75 and 1.00 ml amount of *A. vera* oil treatment. significant group difference was observed in between control and 0.25 ml, control and 0.5 ml, control and 0.75 ml, control and 1.0 ml, 0.25 and 1.0 ml, 0.50 and 1.00 ml and 0.75 and 1.00 ml amount of *A. vera* oil in case of triple treatment (Table-2b, HSD = 0.303).

DISCUSSION

Length of Cocoon: The quantity and the quality of dietary protein has long been considered to be important in the growth of the silkworm [24]. The reserpine of *Rauwolfia serpentina* plays a stimulative role in an increase in the length of cocoon [25]. The silkworm strains have been classified on the basis of cocoon length and other variables [26 - 28]. The economic characters of the silk cocoon were reported to improve by feeding the silkworm with mulberry leaves treated with amino acids [29]. The cocoon length and width are important variables on account of the evolutionary aspects of the silkworm [30, 31]. The variability existed in the polyvoltine germplasm stocks with regard to cocoon length [32]. The number of genes with regards to the expression of cocoon length has been identified and it was found that

not many genes are involved in this [33]. In the present investigation the length of cocoon is increased with increasing the application of amount of *Aloe vera* oil up to 0.75 ml while the cocoon length decreased with an oil up to 1.0 ml showing that the response is largely amount dependent. The higher amount of *A. vera* oil either resulted in the formation of vulnerable larvae or in pupal mortality. This seems to be due to the total disturbance in the endogenous hormone titre and concomitant rearrangements in the tissue metabolic activities. The lower amount of *A. vera* oil may have influenced the metamorphic rhythm as well as economic traits as spinning process.

Volume of Cocoon: The secrets of growth and development of *B. mori* (L) lies in the wealthy nutrition [34]. The variation in the amount of *A. vera* oil and number of larval treatment influenced the volume of *B. mori* cocoon. The topical application of the juvenile hormone or of a structural analog is able to increase the silk production [35]. The reserpine of *Rauwolfia serpentina* plays a stimulative role in an increase in volume of cocoon [25]. Cocoon volume and width variables are important on account of the evolutionary aspects of the silkworm [30, 31] and the variability existed in the polyvoltine germplasm stocks with regard to cocoon volume [32]. The genes with regards to expression of cocoon volume have been identified [33]. Twenty-five eco-races of *Antheraea mylitta* alone reveal interesting variabilities in volume of cocoon [36]. The females spin larger cocoon than the males and thus the female cocoon volume is more than male [36]. The plant extracts could benefit Sericulture by improving the silk yield of *B. mori* and commercial silk production [13]. Thus with the increasing amount of *A. vera* oil from 0.25 to 0.75 ml the volume of cocoon increased. It seems that the increase in the volume of cocoon may be due to the conversion of additional leaf consumed during the extended period into the silk material and the direct stimulatory effect of *A. vera* oil on the protein synthesis in silk gland.

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