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ESSENTIAL OIL (ALOE-VERA) FOR THE IMPROVEMENT OF SILK PRODUCING POTENTIAL IN MULTIVOLTINE MULBERRY SILKWORM (BOMBYX MORI LINN).

PRIYANKA SINGH AND V. B. UPADHYAY*

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

Abstract:

The importance of essential oil (*Aloe-vera*) on the *Bomyx mori* has been proved to be in Sericulture industry. This study was carried out to show the influence of *Aloe-vera* oil on the reelability of filament (%) and denier of filament (d) of *Bombyx mori* eggs. 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 reelability of filament and denier of filament increased with increasing stage of larval treatment and amount of *A. vera* oil up to 0.75 ml, the reelability of filament reached to the maximum level of (84.40±2.821 %) and denier of filament (1.99±0.817 d) in case of triple treated with 0.75 ml *A. vera* oil. Reelability of filament reached to the minimum level of (67.35±1.742 %) and denier of filament (1.26±0.792 d) in case of triple treated with 1.0 ml *A. vera* oil. In conclusion, it may be suggested that, *Aloe-vera* oil in sericulture may be useful for boosting up the Sericulture industry as well as the economy of silkworm rearing.

Keywords- Denier, Reelability, Denier, Silk production, *Aloe-vera* oil, *B. mori*.

Introduction:

The sericulture comprises cultivation of mulberry, silkworm rearing and post cocoon activities leading to production of silk yarn. In recent years, attempts have been made in sericulture to study the effect of temperature (Mishra and Upadhyay, 1995), relative humidity (Upadhyay and Mishra, 2002), ecological factors (Upadhyay *et al*. 2004), egg magnetization (Tripathi and Upadhyay, 2005; Upadhyay and Tripathi, 2006), silk producing potential (Upadhyay and Prasad, 2010), cocoon refrigeration (Upadhyay, *et al.* 2009), phytoecdysteroid hormone (Upadhyay and Pandey, 2012) juvenile hormone analogue and phytoecdysteroid (Srivastava and Upadhyay, 2013), garlic volatile (Fatma, *et al*. 2014) and *aloe vera* oil (Singh, *et al*. 2014) on the performance of silkworm. The plant extracts phytochemicals could benefit sericulture by improving the silk yield of *B. mori* and commercial silk production (Rajasekaragouda *et al.* 1997). The quantity and the quality of dietary protein has long been considered to be important in the growth of the silkworm. The difference in the relative growth rate of *Aloe vera* tonic supplemented larvae from the control observed in the present study indicates that the *Aloe vera* supplementation results in higher protein utilization.

Materials and Methods:

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 (Krishnaswami *et al*. 1973) in the silkworm laboratory , Department of Zoology, DDU Gorakhpur university Gorakhpur. The temperature and relative humidity were maintained in the BOD incubator at 26 ± 1ºC and 80 ± 5% RH respectively until the emergence of moths from the seed cocoons. The newly emerged moths were quickly picked up and kept sex-wise in separate trays to avoid copulation. The whole grainage operation was performed as per description given by Krishnaswami *et al*.**,** (1973). Moth have a tendency to pair

immediately after the emergence, therefore sufficient pairs, each containing one male and one female from newly emerged moth were allowed to mate at 26 ± 1ºC and 80 ± 5%RH in 12 ± 1 hr/day dim light condition. After four hours of mating, the paired moths were decoupled manually. The female moths were allowed for egg laying. After 24 hrs of eggs laying the female moths were individually examined for their disease free ness and after formaline treatment eggs were transferred to the incubator for hatching. After hatching, the larvae were reared on the mulberry leaves given as food in the trays. After completion of 5th instar, the ripe worms ceased feeding and ready for spinning. Small mountages were provided to the ripe worms and thus, sufficient number of cocoons were obtained from the silkworm larvae reared in the laboratory. Further, the cocoons were taken for magnetic exposure.

Adult moths have a tendency to pair immediately after emergence and therefore, the female moths required to copulate with the male moths, were allowed to mate at 26±1ºC and 80±5% 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. After 24 hrs of eggs laying the female moths were individually examined for their disease free ness and after formaline treatment eggs were transferred to the incubator for hatching. After hatching, the larvae were reared on the mulberry leaves given as food in the trays. After completion of 5th instar, the ripe worms ceased feeding and ready for spinning. Small mountages were provided to the ripe worms and thus, sufficient number of cocoons were obtained from the silkworm larvae reared in the laboratory. Further, the cocoons were taken for experiments.

Experimental Design-: To observe the influence of *A. vera* oil on the reelability of filament and denier of filament 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 (**Krishnaswami, *et al*. 1973**). *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 gm 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 ± 1ºC temperature, 80±5% relative humidity and 12 ± 1 hour photoperiod a day.

Reelability of cocoon: For reeling, cocoon was dried in a hot chamber in different batches. The drying chamber was heated up to 120^0 C and then the cocoon was kept into drying chamber. the chamber was maintained at 115^0 C for one hour and after this, temperature was decreased by 15^0 C after every one hour to reach up to 55^0C, so that the temperature profile of 115^0 C, 100^0 C, 85^0 C, 70^0 C, 55^0 C was followed. The cocoons were allowed to cool for half an hour and removed from drying chamber. The total duration of cocoon drying was five and half hours. After this, the cocoons were dipped into hot water to loosen tightly woven filaments. The loosen cocoons were brushed to locate the end of fiber. It is threaded through a porcelain eyelet, and the fiber was reeled on the wheel.

$$\text{Reelability (\%)} = \frac{\text{No. of reeling cocoons}}{\text{No. of feeding ends}} \times 100$$

Where,

No of reeling cocoons = no. of cocoons taken for testing –- no. of converted carry over cocoons

No of feeding ends = no. of casting + no. of carry over cocoons – no. of converted carry over cocoons

Formula used to calculate the converted carry over cocoon and converted unreelable cocoons for multitend test reeling are as follows –

For multi and test reeling machine –

Length (unreelable) = 1.00 new + 0.5 thick + 0.24 middle + 0.06 thin

Weigth (unreelable) = 1.00 new + 0.43 thick + 0.14 middle + 0.03 thin

Length (carry over) = 0.85 thick + 0.24 middle + 0.0.06 thin

Weight (carry over) = 0.43 thick + 0.14 middle + 0.03 thin

For determining the reelability percentage, 300 cocoons (a lot) were taken.

Denier of cocoon: Denier is a unit for the linear mass density of fibres. It is defined as the mass in gram per 9000 meters of filament. Denier is calculated as follows –

$$\text{Denier (d)} = \frac{\text{Wt. of cocoon filament (gm)}}{\text{Length of cocoon filament (m)}} \times 9000$$

Results:

Reelability of filament-The data presented in table 1(a) clearly indicates that change in the amount of *Aloe vera* oil and the number of larval treatment influenced the reelability of filament (%) with the increasing number of treatment from one to three times. The reelability of filament increased in case of 0.25, 0.50, 0.75ml amount of *Aloe vera* oil while in case of 1.00ml amount of *Aloe vera* oil, the reelability of filament increased in single treatment of *Bombyx mori* larvae but further increase in the number of larval treatment caused decline in the reelability of filament. The trend of increase in the reelability of filament with the increasing number of larval treatment has recorded to be almost of similar in case of 0.25, 0.50, 0.75ml amount of *Aloe vera* oil. The maximum reelability of filament was noticed to be 84.40±2.821 (%) in the triple treatment with 0.75ml amount of *Aloe vera* oil. The minimum reelability of filament was recorded 67.35±1.742 (%) in case of triple treatment by 1.00ml amount of *Aloe vera* oil.

Two way ANOVA indicates that the variation in the *Aloe vera* oil treatment significantly ($P_1 < 0.01$) influenced the reelability of filament while the Post hoc test table 1(b) indicates group difference was found in between control and 0.75ml and 0.75 and 1.00ml. in case of double treatment. In triple treatment significant group difference was found in the reelability of filament in between control and 0.75ml, 0.50 and 1.00ml and 0.75 and 1.00ml amount of *Aloe vera* oil. No group difference was found in case of single treatment.

Denier of filament- The data presented in table 2(a) clearly indicates that change in the amount of *Aloe vera* oil and the number of larval treatment influenced the denier of filament (d).With the increasing number of treatment from one to three times. The denier of filament increased in case of 0.25, 0.50, 0.75ml amount of *Aloe vera* oil while in case of 1.00ml amount of *Aloe vera* oil, the denier of filament increased in single treatment of *Bombyx mori* larvae but further increase in the number of larval treatment caused decline in the denier of filament. The trend of increase in the denier of filament with the increasing number of larval treatment has recorded to be almost of similar in case of 0.25, 0.50, 0.75ml amount of *Aloe vera* oil. The maximum denier of filament was noticed to be 1.99±0.817d in the triple treatment with 0.75ml amount of *Aloe vera* oil. The minimum denier of filament was recorded 1.26±0.792d in case of triple treatment by 1.00ml amount of *Aloe vera* oil.

Two way ANOVA indicates that the variation in the *Aloe vera* oil treatment significantly ($P_1 < 0.01$) influenced the denier of filament. table 2(a) while the Post hoc test table 2(b) indicates group difference was found in between control and 0.75ml, 0.50 and 1.00ml and 0.75 and 1.00ml. There was any no significant group difference was found in case of single and double treatment.

Discussion:

Reelability of silk filament: Variation in the amount of *Aloe vera* oil and the number of larval treatment influenced the reelability of silk filament. The minimum reelability of silk filament was noticed with 1.00 ml of *Aloe vera* oil in triple treated larvae, whereas, it reached to the maximum level treated with 0.75 ml of *Aloe vera* oil in triple treated larvae. The administration of plant growth hormone Indloe-3- acetic acid increased the reelability of silk filament (Bhatri and miao, 2003). The phytoecdysteroid administered at different age of 5th instar larvae of *Bombyx mori*, influenced the reelability of silk filament (Nair *et al*., 2005). Methoprene and fenoxycarb treated *Bombyx mori* showed significantly enhanced reelability of silk filament (Mamatha *et al*., 2006). Thyroxine treated *Bombyx mori* shows better reelability (Ahmad *et al*, 2009).

In the present investigation the post cocoon characters positively increased with the increasing amount of *Aloe vera* oil up to 0.75 ml. The stimulatory ability of *Aloe vera* oil on various post cocoon characters contributing to silk yield may be attributed to the synthesis of nucleic acid in the silkworm. The increase in fibroin content may lead to the superior quality of silk. The higher amount of *Aloe vera* oil may cause stress response, resulting in the decline of these commercial characters.

Denier of silk filament- The variation in the amount of *Aloe vera* oil and the number of larval treatment of *Bombyx mori* influenced the denier of silk filament. With the increasing numer of larval treatment from single to triple, the denier of filament of cocoon increased in case of 0.25, 0.50 and0.75 ml treatment of *Aloe vera* oil, while with 1.00 ml, the denier of filament of cocoon increased in single treatment and further decreased with increasing the number of larval treatment. The minimum denier of filament was noticed in case of larvae treated with 1.00 ml of *Aloe vera* oil in triple treated larvae, whereas, the maximum denier of filament was recorded in case of 0.75 ml of *Aloe vera* oil in triple treated larvae. The thyroxine treated mulberry leaf (*Morus multicaulis)* has significant effect on the denier (Ahmad *et al.,* 2009). The administration of plant growth hormone Indloe-3- acetic acid increased the denier value (Bhatri and Miao, 2003). The use of juvenile hormone like methoprene and fenoxycarb during summer season improve the quantitative characters of denier in *Bombyx mori* (Mamtha *et al.*, 2006). The foliar application of different leaves of various micronutrients to mulberry caused significant enhancement in denier in *Bombyx mori*

(Bose and Majumdar, 1996). The uzi-treated cocoons have less denier than the normal cocoon (Mahesha and Honnaiah, 1999). The quantity of mulberry leaves required by a polyvoltine hybrid, BL24XNB4D2 enhanced the denier of silk filament in *Bombyx mori* (Meenal *et al.*, 1999). The phytoecdysteroids when administered at different age of 5th instar larvae of *Bombyx mori* influenced the denier (Nair *et al*., 2005). Methoprene and fenoxycarb treated *Bombyx mori* larvae showed significant enhance in the denier (Mamatha *et al*., 2006). Raw silk is composed of many filaments, variations in thickness of the individual filaments influence the thickness of the thread (Begum *et al*., 2007; Rao *et al*., 1998).

Table 1(a): Effect of essential oil (*Aloe vera* oil) on the reelability of filament (%) of *Bombyx mori*.

Stage of treatment (larval instar)	***Aloe vera* oil applied (ml)**				
	Control (X_1)	**0.25 (X_2)**	**0.50 (X_3)**	**0.75 (X_4)**	**1.00 (X_5)**
Single (5th)	69.30±3.967	72.20±2.543	73.50±2.378	76.65±2.039	70.25±1.585
Double (4th -5th)	69.30±3.967	73.75±2.759	76.90±2.049	80.10±1.786	69.20±1.802
Triple (3rd -4th-5th)	69.30±3.967	76.60±2.663	79.50±2.515	84.40±2.821	67.35±1.742

F_1 = 16.7962 (n_1=4, n_2=38), P_1 < 0.01; F_2 = 2.7305 (n_1=2, n_2=38), not significant.

- Each value represents mean ± S.E. of three replicates.
- **X_1, X_2, X_3, X_4 and X_5** are the mean values of reelability of filament (%) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.

Table 1(b): Post-hoc test showing effect of essential oil (*Aloe vera* oil) on the reelability of filament (%) of *Bombyx mori*.

Mean difference in between groups	Stage of treatment		
	Single	**Double**	**Triple**
$X_1 \sim X_2$	2.90	4.45	7.30
$X_1 \sim X_3$	4.20	7.60	10.20
$X_1 \sim X_4$	7.35	*10.80	*15.10
$X_1 \sim X_5$	0.95	0.10	1.95
$X_2 \sim X_3$	1.30	3.15	2.90
$X_2 \sim X_4$	4.45	6.35	7.80
$X_2 \sim X_5$	1.95	4.55	9.25
$X_3 \sim X_4$	3.15	3.20	4.90
$X_3 \sim X_5$	3.25	7.70	*12.15
$X_4 \sim X_5$	6.40	*10.90	*17.05

Honesty significant difference (HSD) $= q\sqrt{\frac{\text{MS within}}{n}}$

$= {}^{5.05}\sqrt{\frac{12.7812}{3}}$

$= 10.4232$

MSE = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of reelability of filament (%) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.

Table 2(a): Effect of essential oil (*Aloe vera* oil) on the denier of filament (d) of *Bombyx mori*.

Stage of treatment (larval instar)	*Aloe vera* oil applied (ml)				
	Control (X_1)	0.25 (X_2)	0.50 (X_3)	0.75 (X_4)	1.00 (X_5)
Single (5^{th})	1.41±0.779	1.55±0.807	1.63±0.759	1.67±0.763	1.38±0.773
Double (4^{th} -5^{th})	1.41±0.779	1.66±0.989	1.72±0.779	1.80±0.836	1.35±0.784
Triple (3^{rd} -4^{th}-5^{th})	1.41±0.779	1.71±0.761	1.83±0.805	1.99±0.817	1.26±0.792

F_1 =12.8875 (n_1=4, n_2=38), P_1 < 0.01; F_2 =1.5514 (n_1=2, n_2=38), not significant.

- Each value represents mean ± S.E. of three replicates.
- **X_1, X_2, X_3, X_4 and X_5** are the mean values of denier of filament (d) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.

Table 2(b): Post-hoc test showing effect of essential oil (*Aloe vera* oil) on the denier of filament (d) of *Bombyx mori*.

Mean difference in between groups	Stage of treatment		
	Single	Double	Triple
X_1 ~ X_2	0.14	0.25	0.30
X_1 ~ X_3	0.22	0.31	0.42
X_1 ~ X_4	0.26	0.39	*0.58
X_1 ~ X_5	0.03	0.06	0.15
X_2 ~ X_3	0.08	0.06	0.12
X_2 ~ X_4	0.12	0.14	0.28
X_2 ~ X_5	0.17	0.31	0.45
X_3 ~ X_4	0.04	0.08	0.16
X_3 ~ X_5	0.25	0.37	*0.57
X_4 ~ X_5	0.29	0.45	*0.73

Honesty significant difference (HSD) $= q\sqrt{\frac{\text{MS within}}{n}}$

$= {}^{5.05}\sqrt{\frac{0.0304}{3}}$

$= 0.5075$

MSE = Mean square value of ANOVA Table

q = Studentized range static

n = No. of replicates

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of denier of filament (d) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.

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