Bioactive influence of essential oil (Aloe-vera) on the number of fertilized eggs (%) and incubation period of multivoltine mulberry silkworm Bombyx mori Linn.

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Abstract: The evaluation 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 number of fertilized eggs (%) and incubation period of Bombyx mori eggs. In 0.25, 0.5 and 0.75 ml. amount of Aloe-vera oil, the incubation period decreased slowly and slowly, in single (treated the 5th instar larvae only one times) and double treatment (in first treatment treated the 4th instar just before two days of 4th moulting instar, second treatment for the same larvae was given to the 4th instar just before two days of spinning) but 1.00 ml. amount of Aloe vera oil in all the treatment caused considerable increase in the incubation period of eggs. The minimum incubation period was noticed (8.29±1.357 days) in case of double treatment by 0.75 ml. amount of Aloe-vera oil. Maximum number of fertilized eggs was noticed (81.00±2.239 %) in case of double treatment by 0.75 ml. amount of Aloe-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- Aloe-vera oil, 4th instar larvae, Eggs, Moulting, Incubation period, Rearing, Sericulture.

INTRODUCTION

The silkworm, *Bombyx mori* L. is a typical monophagous insect and mulberry (Morus spp.) leaf is its sole food. Nutrition of silkworm is sole factor which almost individually augment quantity and quality of silk. Man has immensely benefited from the silk produced by silkworms and subsequently researchers have always been trying to unveil the factors that can be manipulated to the benefit of the silkworm rearers. The per cent fertilized egg and incubation period are important factors that directly influence the production of good cocoon. *Aloe-Vera* plant contains 99.5% water and 0.013% protein which play important role in the nutrition of silk worm and silk production. Thus *A. vera* oil is beneficial for silkworm. In recent years, attempts have been made in sericulture to study the effect of temperature **[1],** relative humidity **[2]**, ecological factors **[3],** egg magnetization **[4]; [5],** cocoon magnetization **[6],** cocoon refrigeration **[7],** 20-hydroxyecdysone hormone **[8]**, phytoecdysteroid hormone **[9] [10],** garlic volatile **[11]** and *aloe vera* oil **[12]** on the performance of silkworm. The plant extracts phytochemicals could benefit sericulture by improving the silk yield of *B. mori* and commercial silk production **[13].** 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

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 **[14]** in the silkworm laboratory , Department of Zoology, DDU Gorakhpur university Gorakhpur. The temperature and relative humidity were maintained at 26±1ºC and 80±5% RH, 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 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.

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 ± 1ºC and 80 ± 5%

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 fecundity and hatchability 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 **[14].** *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.

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. 100 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.

Per cent Fertilized eggs- For determining the effect of *Aloe-vera* oil on the per cent fertilized eggs, obtained from the moths of treated larvae were considered and the eggs thus obtained, were incubated at optimum conditions of temperature, relative humidity and photoperiod of 26±1ºC, 80±5% and 12±1 h, respectively. At the head pigmentation stage the eggs were counted. Thirty layings (3 batches of 10 laying in each batch) were counted for each replicate. The per cent of the fertilized eggs was calculated as follows-

$$\text{Per cent fertilized eggs} = \frac{\text{No. of eggs fertilized*}}{\text{Total no. of eggs}} \times 100$$

*Eggs were counted at the head pigmentation stage, i.e. eggs having black spots were fertilized eggs.

Incubation period of egg- The eggs (obtained after treatments) were transferred chronically to BOD incubator maintained at the optimum condition of 26±1ºC temperature, 80±5% RH and 12 h of photoperiod a day. The time required for the incubation before the hatching of larvae was calculated for each set of experiment separately. The average of eggs was counted from 8 pm on the day of decoupling. Three replicates of 10 laying, in each replicate, were made. The average time taken for incubation by the eggs of different experimental conditions was calculated by taking the mean value of the data obtained.

RESULTS

Per cent fertilized eggs- The data presented in table 4a clearly indicates that variation in the amount of *Aloe vera* oil and the number of treatment influenced the per cent fertilized eggs (%) of adult female moth. With the increasing number of treatment from one to three times. The percent fertilized eggs 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 percent fertilized eggs increased in single treatment of *Bombyx mori* larvae but further increase in the number of larval treatment caused decline in the percent fertilized eggs. The trend of increase in the fecundity with the increasing number of treatment has recorded to be almost of similar in case of 0.25, 0.50, 0.75ml amount of *Aloe vera* oil. The maximum percent fertilized egg of adult moth was noticed to be 81.00±2.239 % in the triple treatment with 0.75ml amount of *Aloe vera* oil. The minimum percent fertilized egg was recorded 60.50±1.014 % in case of triple treatment by 1.0 ml 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 percent fertilized eggs of adult female moth. table 4a while the Post hoc test table 4b indicates no group difference was found in case of single treatment. In case of double treatment significant group difference was found in between 0.25 and 1.00ml, 0.25 and 1.00ml, 0.50 and 1.00ml and 0.75 and 1.00ml amount of *Aloe vera* oil. In case of triple treatment significant group difference was found in between control and 1.00 ml, 0.25 and 1.00ml, 0.50 and 1.00ml and 0.75 and 1.00ml.

Incubation period- The data presented in table 5a clearly indicates that variation in the amount of *Aloe vera* oil and the number of treatment influenced the incubation period of egg (days) with the increasing number of larval treatment from one to three times. The incubation period of egg decreased in case of 0.25, 0.50, 0.75ml amount of *Aloe vera* oil but triple treatment caused notable increase in the incubation period in all the above amount of *Aloe vera* oil. 1.00ml amount of *Aloe vera* oil treatment caused notable increase in the incubation period of egg with the increase in number of treatment from single to triple. The trend of decrease in the incubation period of egg with the increasing number of treatment has been recorded to be almost same in case of 0.25, 0.50 and 0.75ml. amount of *Aloe vera* oil treatment. The minimum incubation period was noticed to be 8.29±1.357 days in case of triple treatment by 0.75ml amount of *Aloe vera* oil and the maximum 10.76±1.910 days was recorded 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 incubation period of egg of adult female moth table 5a while the Post hoc test table 5b indicates any no significant group difference was found.

DISCUSSION

Per cent fertilized egg- Variation in the amount of *Aloe vera* oil and number of larval treatment notably influenced the per cent fertilized eggs. With the increasing number of larval treatment, the per cent fertilized eggs increased in 0.25, 0.50 and 0.75 ml amount of *Aloe vera* oil. The per cent fertilized eggs increased with the increasing amount of *Aloe vera* oil from 0.25 to 0.75 ml. Genetic diversity among silkworm breeds may cause low egg recovery under different environmental conditions. The egg laying capability of *B. mori* has been noticed to be influenced by the genotype of silkworm line and rearing temperature. The occurance of unfertilized eggs was more common in summer as compared to other seasons **[15].** Variation in refrigeration period of silkworm eggs caused considerable influence on the pupal duration of *Bombyx mori.* **[16].**

Incubation period of eggs- The change in the amount of *Aloe vera* oil and the number of larval treatment, considerably influenced the incubation period of *Bombyx mori* eggs. In case of 0.25, 0.50 and 0.75 ml amount of *Aloe vera* oil, the incubation period of eggs decreased slowly. The trend of variation of the incubation period in 0.25, 0.50 and 0.75 ml amount is almost of similar pattern with varying number of larval treatment. In insects, the steroid hormone 20- hydroxyecdysone (20 E) plays a major role in activating vitellogenesis, a process required for egg development **[17].**

CONCLUSION

Thus it may be inferred that treatment with *Aloe vera* oil up to 0.75 ml may influence the embryonic development positively resulting in the enhanced rate of cleavage, causing reduction in the embryonic duration. The higher amount may cause inhibitory effects on the cleavage; hence, the rate of cleavage declined causing prolongation in the incubation period of eggs.

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Table 1a: Effect of essential oil (*Aloe vera* oil) on the number of fertilized eggs (%) of *Bombyx mori*.

Stage of treatment (larval instar)	Control (X_1)	*Aloe vera* oil applied (ml)			
		0.25 (X_2)	0.50 (X_3)	0.75 (X_4)	1.00 (X_5)
Single (5th)	72.00±2.712	72.50±2.888	75.00±1.712	75.50±1.933	67.50±2.517
Double (4th -5th)	72.00±2.712	74.50±1.337	79.00±1.289	79.50±1.053	64.50±1.933
Triple (3rd -4th-5th)	72.00±2.712	76.50±2.470	80.50±1.953	81.00±2.239	60.50±1.014

F_1 = 3.3142 (n_1=4, n_2=38), P_1 < 0.05; F_2 = 0.4405 (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 number of fertilized eggs (%) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.

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

Mean difference in between groups	Stage of treatment		
	Single	**Double**	**Triple**
$X_1 \sim X_2$	0.50	2.50	4.50
$X_1 \sim X_3$	3.00	7.00	8.50
$X_1 \sim X_4$	3.50	7.50	9.00
$X_1 \sim X_5$	4.50	7.50	*11.50
$X_2 \sim X_3$	2.50	4.50	4.00
$X_2 \sim X_4$	3.00	5.00	4.50
$X_2 \sim X_5$	5.00	*10.00	*16.00
$X_3 \sim X_4$	0.50	0.50	0.50
$X_3 \sim X_5$	7.50	*14.50	*20.00
$X_4 \sim X_5$	8.00	*15.00	*20.50

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

$= {}^{5.05}\sqrt{\frac{9.5639}{3}}$

$= 9.0168$

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 number of fertilized eggs (%) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.

Table 2a: Effect of essential oil (*Aloe vera* oil) on the Incubation period (days) of *Bombyx mori*.

Stage of treatment (larval instar)	Control (X_1)	*Aloe vera* oil applied (ml)			
		0.25 (X_2)	0.50 (X_3)	0.75 (X_4)	1.00 (X_5)
Single (5th)	10.24±1.861	10.00±1.867	9.76±1.899	8.95±1.333	10.31±1.474
Double (4th -5th)	10.24±1.861	9.62±1.567	9.30±1.279	8.75±1.037	10.62±1.132
Triple (3rd -4th-5th)	10.24±1.861	9.46±1.368	8.88±1.053	8.29±1.357	10.76±1.910

F_1 =32.6618 (n_1=4, n_2=38), P_1 < 0.01; F_2 =1.1919 (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 incubation period (days) 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* oil) on the Incubation period (days) of *Bombyx mori*.

Mean difference in between groups	Stage of treatment		
	Single	Double	Triple
$X_1 \sim X_2$	0.11	0.21	0.33
$X_1 \sim X_3$	0.23	0.38	0.78
$X_1 \sim X_4$	1.29	0.53	0.81
$X_1 \sim X_5$	0.07	0.02	0.02
$X_2 \sim X_3$	0.12	0.17	0.45
$X_2 \sim X_4$	1.05	0.32	0.48
$X_2 \sim X_5$	0.04	0.19	0.35
$X_3 \sim X_4$	0.81	0.15	0.03
$X_3 \sim X_5$	0.16	0.36	0.80
$X_4 \sim X_5$	1.36	0.50	0.83

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

$= {}^{5.05}\sqrt{\frac{1.8239}{3}}$

$= 3.9375$

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 incubation period (days) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.