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Biological Importance of Essential Oil on the Duration and Survival of Pupae of *Bombyx mori* Nistari.

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Abstract: India is the second largest producer of raw silk after china and the biggest consumer of raw silk and silk fabrics. *Bombyx mori* is an important economic insect because it converts leaf protein in to silk protein. Present study was carried out to evaluate the effect of essential oil on the pupal length and pupal duration of *B. mori* pupae.The experiments were performed with *Aloe vera* essential oil viz. 0.25, 0.50, 0.75 and 1.00 ml with respect to the single, double and triple treatment of *B. mori* larvae. The survival of larvae increased with increasing the number of larval treatment by 0.25, 0.50 and 0.75 ml *A.vera* essential oil. The minimum pupal duration (8.126±0.128 days) was recorded in case of triple treatment by 0.75 ml *A. vera* essential oil. The maximum level of survival of pupae (94.186±0.758%) was noticed in case of triple treatment with 0.75 ml *A. vera* essential oil. It is suggested that administration of *A.vera* essential oil may also help to device improvement in the rearing program of multivoltine mulberry silkworm to increase the quality and quantity of silk at commercial scale.

Key words: Mulberry Silkworm · *Aloe vera* · Stage of Treatment · *Morus* Plant · Pupae

INTRODUCTION

Sericulture is an integral part of the rural economy in an agrarian country like India, with high employment potential and economic benefits to agrarian families. In India, Uttar Pradesh is a developing mulberry silk producing state where the Tarai Belt is gaining a leading status in the extension of sericulture industry. *Bombyx mori* race nistari is a resistant variety of multivoltine mulberry silkworm which contributes up to a great extent in the commercial production of cocoon in varying ecological conditions in our country. The larval stages of *B. mori* are one of the typical leaf feeders, since a diet of which the chemical content is completely known, both quantitatively and qualitatively. The ultimate aim of sericulture industry is the production of quality seed cocoons i.e. raw silk as per demand. The pupal performance is a very important factor that directly influences the production of cocoon. In order to increase production and quality of raw silk several efforts have been made to study the effect of ecological factors [1], refrigeration of egg [2], cocoon [3, 4], cocoon magnetization [5], 20-hydroxyecdysone hormone [6], phytoecdysteroid [7], linseed and hemp oil [8] and garlic volatile exposure duration [9,10] on the performance of *B. mori. Aloe vera* essential oil also influenced on the performance of silkworm [11-13]. *Aloe vera* herbal tonic Alloe [14] and Aloe tonic treated mulberry leaves [15] influenced the larval growth of *B. mori*. Plant extracts and their essential oils are interesting as sources of natural products for decades [16]. Many kinds of essential oils have been screened for their potential uses for food preservation, aromatherapy and fragrance industry [17]. Beneficial effects of *A. vera* (Hindi- Gikanvar or Ghrita kumari) in human and laboratory animals are contributed to the promotion of immune system, analgesic, anti-inflammatory, wound healing and anti-tumor activities as well as antiviral, antibacterial and antifungal properties [18]. *Aloe vera* products are also used in medicine folk, cosmetics, supplement and food material [19]. *Aloe vera* is composed of 75 potentially active compounds: vitamins, enzymes, minerals, sugars, lignin, salicylic acids and amino acids [20], aloin and saponin [21]. Thus an attempt has been made to study the *A. vera* essential oil treated mulberry leaves on the pupal performance of *B. mori* Linn.

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MATERIALS AND METHODS

The seed cocoons of multivoltine mulberry silkworm (*Bombyx mori* nistari) were obtained from the silkworm grainage. Directorate of sericulture, Behraich Uttar Pradesh and were maintained in the plywood trays (23 x 20 x 5cm) under the ideal rearing conditions in the silkworm laboratory, Department of Zoology, DDU Gorakhpur University Gorakhpur. The temperature and relative humidity were maintained at 26 ± 1°C and 80 ± 5% RH, respectively till 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 male moths were smaller in size but more active than the female months which were comparatively larger and less active. The whole grainage operation was performed as per description given by Krishnaswamy *et al.* [22].

Moths have a tendency to pair immediately after emergence and, therefore, the female moths required to copulate with the male moths, were allowed their mates for copulation. Sufficient pairs, each containing one male and one female from newly emerged moths were allowed to mate at 26±1°C 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 fore finger. The male moths were discarded while the female moths were allowed to lay eggs. After 24 hours of egg laying, the female moths were individually examined for their disease freeness.

The disease free layings (D.F.L's), thus prepared, were treated with 2% formaline for 15 minutes to increase the adhesiveness of eggs on the paper sheet and surface disinfection. Thereafter, the egg sheets, with egg laid on, were thoroughly washed with running water to remove formaline and the eggs were dried in shade. The dried eggs were transferred to the incubator for hatching. After hatching, the larvae were reared on the mulberry leaves given as food in the trays. Further, the 3rd instar larvae were taken for experiment.

Experimental Design: To observe the influence of *A. vera* essential oil on the pupal performance of *B. mori,* the experiment was performed with different amount of *Aloe vera* essential oil with respect to the treatment of 3rd, 4th and 5th instar larvae. *A. vera* essential oil purchased from the Katyani Exports Delhi, India. Four amount of *A. vera* essential oil viz, 0.25, 0.50, 0.75 and 1.00 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 sets of experiment were designed viz, single, double and triple treatment of larvae. All the experiments were conducted in the BOD incubator. The experiment was conducted on normal rearing condition i.e. 26±1°C temperature, 80±5% relative humidity and 12±1 hour photoperiod a day.

Single Treatment: Single treatment of larvae was performed with the 5th instar larvae just before two days of the beginning of larval spinning. One hundred larvae were taken out from the BOD incubator and the mulberry leaf treated with 0.25 ml of *A. vera* essential oil was given as food. Further, the treated larvae were given normal mulberry leaf for food.

Double Treatment: Double treatment of larvae was started from the final stage of 4th instar larvae. In the first treatment, one hundred larvae of 4th instar were treated just before two days of 4th moulting, by providing treated mulberry leaf as food with 0.25 ml of *A. vera* essential 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. Thus, in double treatment, 4th and 5th instar larvae were treated.

Triple Treatment: For triple treatment, the 3rd instar larvae just before 3rd moulting were separated from BOD incubator. In the first treatment, one hundred 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 incubator for further rearing. Third treatment was given to 5th instar larvae, two days before the start of spinning by providing mulberry leaf treated with 0.25 ml of *A. vera* essential oil as food. Thus, in the triple treatment 3rd, 4th and 5th instar larvae were treated.

Similar experiments were performed by 0.50, 0.75 and 1.00 ml of *Aloe vera* essential oil. A control set was always maintained with each set of experiment.

For Determining the Pupal Duration: The time required from the third day of spinning (formation of pupae) to the emergence of moth was considered for pupal duration. For this purpose, 75 cocoons along with their pupae were taken for observation. Three replicates of each experiment were made.

For Determining the Survival of Pupae: For determining the survival percentage of pupae 75 pupae were taken under the observation.The number of pupae emerged as moth was counted for calculating the survival of pupae as following.

$$\text{Per cent survival of pupae} = \frac{\text{No. of moths emerged}}{\text{No. of pupae were taken for observation}} \times 100$$

RESULTS

Pupal Duration: It is clear from the Table-1a that changes in the *Aloe vera* essential oil amount and the number of larval treatment influenced the pupal duration. With the increase in number of larval treatment by *A. vera* essential oil from one to three times, the pupal duration decreased in case of 0.25, 0.50 and 0.75 ml treatment, while treatment with 1.00 ml *A. vera* essential oil caused notable increase in the pupal duration with increase in the number of larval treatment from single to triple. The trend of decrease in the pupal duration with the increasing number of larval treatment has been recorded to be almost same in case of 0.25, 0.50 and 0.75 ml *A. vera* essential oil treatment. The minimum pupal duration was recorded to be 8.126±0.128 days (22.06% decreased as compare to control) in case of triple treatment of larvae by 0.75 ml of *A. vera* essential oil and the maximum larval duration 12.037±0.307 days was recorded in case of triple treatment of larvae by 1.00 ml *A. vera* essential oil.

Two-way ANOVA indicates that variation in the *A. vera* essential oil amount significantly ($P_1<0.01$) influenced the pupal duration, while variation in number of larval treatment has no significant effect (Table-7a). The Post-hoc test (Table-1b, HSD=1.527) shows significant group difference in the pupal duration in between control and 0.75 ml, 0.25 and 1.00 ml, 0.50 and 1.00 ml and, 0.75 and 1.00 ml *Aloe vera* essential oil in case of double treatment of larvae. In the triple treatment of larvae, significant group difference in the larval duration was noticed in between control and 0.50 ml, control and 0.75 ml, control and 1.00 ml, 0.25 and 1.00 ml, 0.50 and 1.00 ml and 0.75 and 1.00 ml of *A. vera* essential oil treatment. In case of single treatment there was no significance group difference.

Survival of Pupae: The data given in Table-2a is indicative of the fact that variation in the *A. vera* essential oil amount and the number of larval treatment influenced the survival of pupae. With the increase in number of larval treatment by *A. vera* essential oil from one to three times, the survival of pupae increased in case of 0.25, 0.50 and 0.75 ml of *A. vera* essential oil treatment, while treatment with 1.00 ml *A. vera* essential oil caused decline in the survival of pupae with increase in the number of larval treatment from single to triple. Increase in the survival per cent of pupae with the increasing number of larval treatment has been recorded to be almost of similar trend in case of 0.25, 0.50 and 0.75 ml *A. vera* essential oil treatment. The maximum survival of pupae was recorded to be 94.186±0.758% (11.74% increased as compare to control) in case of triple treatment of larvae by 0.75 ml of *A. vera* essential oil and the minimum survival of pupae 75.863± 1.046% was recorded in case of triple treatment of larvae by 1.00 ml *A. vera* essential oil.

Two-way ANOVA shows that variation in the *A. vera* essential oil amount caused significant ($P_1<0.01$) influence on the survival of pupae, while variation in number of larval treatment did not cause significant effect (Table-8a). The Post-hoc test (Table-2b, HSD=7.072) indicates

Table 1a: Effect of *Aloe vera* essential oil treatment on the pupal duration (day) of *Bombyx mori*.

Stage of treatment (larval instar)	*Aloe vera* essential oil applied (ml)				
	Control (X_1)	0.25 (X_2)	0.50 (X_3)	0.75 (X_4)	1.00 (X_5)
Single (5th)	10.426±0.163 (100)	10.253±0.126 (98.34)	10.025±0.195 (96.15)	9.512±0.132 (91.23)	10.958±0.129 (105.10)
Double (4th -5th)	10.426±0.163 (100)	9.832±0.181 (94.30)	9.360±0.115 (89.78)	8.840±0.299 (84.79)	11.518±0.281 (110.47)
Triple (3rd -5th)	10.426±0.163 (100)	9.556±0.154 (91.66)	8.785±0.437 (84.26)	8.126±0.128 (77.94)	12.037±0.307 (115.45)

•F_1 = 34.5270 (n_1=4, n_2=38), P < 0.01; F_2 = 2.7591 (n_1=2, n_2=38), not significant. (F_1= *Aloe vera* essential oil applied and F_2= Stage of treatment)

•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 pupal duration in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* essential oil treatment, respectively.

•Figures in parentheses indicate percent value when control was taken as 100%.

Table 1b: Post-hoc test showing effect of *Aloe vera* essential oil treatment on the pupal duration of *Bombyx mori*.

	Stage of treatment		
Mean difference in between groups	Single	Double	Triple
$X_1 \sim X_2$	0.173	0.594	0.870
$X_1 \sim X_3$	0.401	1.066	*1.641
$X_1 \sim X_4$	0.914	*1.586	*2.300
$X_1 \sim X_5$	0.532	1.092	*1.611
$X_2 \sim X_3$	0.228	0.472	0.771
$X_2 \sim X_4$	0.741	0.992	1.430
$X_2 \sim X_5$	0.705	*1.686	*2.481
$X_3 \sim X_4$	0.513	0.520	0.659
$X_3 \sim X_5$	0.933	*2.158	*3.252
$X_4 \sim X_5$	1.446	*2.678	*3.911

Honesty significant difference (HSD = $q\sqrt{\frac{MS\,within}{n}} = 5.05\sqrt{\frac{0.274}{3}} = 1.527$

MSE = Mean Square Error from ANOVA table

q = Value from studentized range table

n = No. of replicates per treatment

* = Shows significant group difference

X_1, X_2, X_3, $X_4$and $X_5$are mean values of pupal duration in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* essential oil treatment, respectively.

Table 2a: Effect of *Aloe vera* essential oil treatment on the survival per cent of *Bombyx mori* pupae.

Stage of treatment (larval instar)	*Aloe vera* essential oil applied (ml)				
	Control (X_1)	0.25 (X_2)	0.50 (X_3)	0.75 (X_4)	1.00 (X_5)
Single (5th)	84.293±1.189 (100)	87.289±1.198 (103.55)	88.167±0.603 (104.60)	89.285±1.208 (105.92)	82.126±1.181 (97.43)
Double (4th -5th)	84.293±1.189 (100)	89.873±1.349 (106.62)	89.892±1.015 (106.64)	91.216±0.660 (108.21)	79.856±0.464 (94.74)
Triple (3rd -5th)	84.293±1.189 (100)	90.663±0.735 (107.56)	92.303±0.996 (109.50)	94.186±0.758 (111.74)	75.863±1.046 (90.00)

•$F_1 = 39.2416$ (n_1=4, n_2=38), $P < 0.01$; $F_2 = 0.9929$ (n_1=2, n_2=38), not significant. (F_1= *Aloe vera* essential oil applied and F_2= Stage of treatment)

•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 survival per cent of pupae in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* essential oil treatment, respectively.

• Figures in parentheses indicate percent value when control was taken as 100%.

Table 2b: Post-hoc test showing effect of *Aloe vera* essential oil treatment on the survival of *Bombyx mori* pupae.

	Stage of treatment		
Mean difference in between groups	Single	Double	Triple
$X_1 \sim X_2$	2.996	5.580	6.370
$X_1 \sim X_3$	3.874	5.599	*8.010
$X_1 \sim X_4$	4.992	6.923	*9.893
$X_1 \sim X_5$	2.167	4.437	*8.430
$X_2 \sim X_3$	0.878	0.019	1.640
$X_2 \sim X_4$	1.996	1.343	3.523
$X_2 \sim X_5$	5.163	*10.017	*14.800
$X_3 \sim X_4$	1.118	1.343	1.883
$X_3 \sim X_5$	6.041	*10.036	*16.440
$X_4 \sim X_5$	*7.159	*11.360	*18.323

Honesty significant difference (HSD) = $q\sqrt{\frac{MS\,within}{n}} = 5.05\sqrt{\frac{5.882}{3}} = 7.072$

MSE = Mean Square Error from ANOVA table

q = Value from studentized range table

n = No. of replicates per treatment

* = Shows significant group difference

X_1, X_2, X_3, X_4 and X_5 are mean values of survival of pupae in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* essential oil treatment, respectively.

significant group difference in the survival of pupae in between 0.75 and 1.00 ml *A. vera* essential oil in case of single treatment. In case of double treatment of larvae, significant group difference in the survival of pupae was noticed in between 0.25 and 1.00 ml, 0.50 and 1.00 ml and 0.75 and 1.00 ml *A. vera* essential oil. In the triple treatment of larvae, significant group difference in the survival of pupae was noticed in between control and 0.50 ml, control and 0.75 ml, control and 1.00 ml, 0.25 and 1.00 ml, 0.50 and 1.00 ml and 0.75 and 1.00 ml of *A. vera* essential oil treatment.

DISCUSSION

The change in the *Aloe vera* essential oil amount and the number of larval treatment influenced the pupal duration of *Bombyx mori*. The pupal duration of *B. mori* has been noticed to vary due to change in the varieties of mulberry, given as food to larvae [23]. The low magnetic field caused stimulatory effect, whereas, higher magnetic field caused inhibitory effect on pupal duration of *Drosophila melanogaster* [24]. The magnetic field has been noticed to be responsible for the activation of enzyme (Carboxymutase and Catalase) activities in the biological system [25]. Dietary administration of the vertebrate sex hormone menstral and northindrone on the silkworm reduced the pupal duration [26] whereas, juvenile hormone secreted by corpora allota is responsible for preventing the metamorphosis [27]. The treatment with synthetic juvenoid R-394 caused the prolongation in the pupal duration [28], while Juvenile hormone mimic R-394, when topically applied on the abdomen tergum of silkworm, improved the pupal duration [29]. Treatment with 60% phytoecdystreroid reduced pupal duration in silkworm [7]. The ecological factors [1] and referegeration of cocoon [3,4] caused increase in pupal duration of silkworm. Larvae fed on soybean and mushroom diet gave the shortest pupal duration in *B. mori* [30].

The survival per cent of *B. mori* pupae increased gradually with the increase in number of larval treatment by *A. vera* essential oil from one to three times in case of 0.25, 0.50 and 0.75 ml of *A. vera* essential oil. The survival and development of insects are at the prevailing ecological conditions and to a certain extent to their genetic built up [22, 31, 32]. High temperature during the later developmental stage considerably reduced the pupation and survival rate of pupae [33], while the physiological changes due to variation in the rearing temperature [2] influenced the survival of pupae in *B. mori.* Refrigeration of cocoon affected the survival of silkworm pupae [3, 4]. The administration of riboflavin [34] and folic acid significantly increased the survival per cent of silkworm pupae [35-37]. The insect growth regulator fenoxycrab affect in a dose-depend manner that increased mortality during larval-pupal transformation in *B. mori* [38]. Topical application of juvenile hormone mimics R-394 [29] and double treatment with 60% phytoecdystreroid increased the survival of pupae in silkworm [7].

The above observation indicates that pupal duration decreased with increasing *Aloe vera* essential oil amount up to 0.75 ml with triple treatment of larvae and survival of pupae increased with the increase in the number of treatment with different amount of *A. vera* essential oil. Treatment of *B. mori* larvae with different amount of *A. vera* essential oil may cause certain ultra structural changes during larval and pupal stages that increases the enzyme activity resulting in the increase of the general metabolic rate leading to survival per cent of pupae.

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