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Biological Significance of Essential Oil on the Duration and Survival of Larvae of Multivoltine Mulberry Silkworm

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Abstract: The silkworm *Bombyx mori* L., an economic sericigenous insect, show high activity after feeding on the treated mulberry leaves with essential oils. 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. Variation in the *A. vera* essential oil amount influenced the duration and survival % of larvae. The survival of larvae increased with increasing the number of larval treatment by 0.25, 0.50 and 0.75 ml *Aloe vera* essential oil. The maximum level of survival of larvae (96.427±0.681%) was noticed in case of triple treatment with 0.75 ml *A. vera* essential oil. The minimum larval duration (21.436±0.313 days) was recorded in case of triple treatment by 0.75 ml *Aloe vera* essential oil, showing good development of larvae. It is suggested that supplementation of *Aloe vera* essential oil may also help to device improvement in the rearing programme of multivoltine mulberry silkworm to increase the quality and quantity of silk and boosting up the sericulture industry as well as the economy of silkworm rearing.

Key words: *Bombyx mori* · *Morus alba* · *Aloe vera* · Sericulture · Larval Traits

INTRODUCTION

In India, sericulture is not only a tradition but also a living culture. It provides income and employment to the rural farmers with small land-holding. India is the second largest producer of mulberry silk next only to China. In recent past, several attempts have been made to develop successful silk production. Silkworm is a highly sensitive insect and responds sharply to changes in the feed quality. The effects of various kinds of dietary protein on growth of the silkworm *Bombyx mori* L. were determined using semi-synthetic diets. Plant extracts and their essential oils are interesting as sources of natural products for decades [1]. Many kinds of essential oils have been screened for their potential uses for food preservation, aromatherapy and fragrance industry [2]. Beneficial effects of *Aloe 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 [3]. *Aloe vera* is composed of 75 potentially active compounds: vitamins, enzymes, minerals, sugars, lignin, salicylic acids and amino acids [4], aloin and saponin [5]. *Aloe vera* products are also used in medicine folk, cosmetics, supplement and food material [6]. The larval performance is an important factor that directly influences the production of good cocoon. In recent years efforts have been made in sericulture to study the effect of temperature [7], relative humidity [8, 9], ecological factors [10], egg refrigeration [11], cocoon refrigeration [12], cocoon magnetization [13], 20-hydroxyecdysone hormone [14], phytoecdysteroid [15] and linseed and hemp oil [16] on the performance of *B. mori*. *Aloe vera* essential oil also influenced on the performance of silkworm [17, 18]. *Aloe vera* herbal tonic 'logen' [19], Alloe [20] and Aloe tonic treated mulberry leaves [21] influenced the larval growth of *B. mori*. Thus an attempt has been made to study the *A. vera* essential oil treated mulberry leaves on the larval 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*; 1973 [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 larval performance of *B. mori,* the experiment was performed with different amount of *A. 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 gm 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 *Aloe 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 *Aloe vera* essential oil as food. Thus, in the triple treatment 3rd, 4thand 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 Larval Duration: The time required from the hatching of larvae to the third day of spinning, by the fifth instars larvae, was considered for larval duration. For this purpose, 90 larvae were taken for observation. Three replicates of each experiment were made.

For Determining the Survival of Larvae: For determining the survival of larvae 90 larvae were taken under the observation.The number of larvae which attained the pupal stage was counted for the calculation of the survival of larvae as following:

$$Percent\ survival\ of\ larvae = \frac{\text{No. of larvae pupated}}{\text{No of 3rd instar larvae taken for observation}} \times 100$$

RESULTS

Larval Duration: The data given in Table-1a is indicative of the fact that changes in the *A. vera* essential oil amount and the number of larval treatment influenced the larval duration. With the increase in number of larval treatment by *A. vera* essential oil from one to three times, the larval duration decreased in case of 0.25, 0.50 and 0.75 ml of *A. vera* essential oil treatment, but treatment with 1.00 ml *A. vera* essential oil caused increase in the larval duration with increase in the number of larval treatment from single to triple. The trend of decrease in the larval duration with the increase in number of larval treatment has been recorded to be almost similar in case of 0.25, 0.50 and 0.75 ml *A. vera* essential oil treatment. The minimum larval duration was recorded to be 21.436±0.313 days (7.55% 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 25.035±0.330 days 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 significantly (P_1<0.01) influenced the larval duration while variation in number of larval treatment did not cause significant effect (Table 1). The Post-hoc test (Table-1b, HSD=1.875) indicates significant group difference in the larval duration in between 0.75 and 1.00 ml *A. 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 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. No significant group difference was noticed in case of single treatment.

Survival of Larvae: The data given in Table-2a indicates that variation in the *A. vera* essential oil amount and the number of larval treatment influenced the survival of larvae. With the increase in number of larval treatment by *A. vera* essential oil from one to three times, the survival of larvae 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 notable decrease in the survival of larvae with increase in the number of larval treatment from single to triple. The pattern of increase in the survival of larvae with the increasing number of larval treatment has been recorded to be almost similar in case of 0.25, 0.50 and 0.75 ml *A. vera* essential oil treatment. The maximum survival of larvae was recorded to be 96.427±0.681% (26.46% 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 larvae 56.858±0.942 % 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 survival of larvae, while variation in number of larval treatment has no significant influence on the survival of larvae (Table-2a). The Post-hoc test (Table-2b, HSD=14.965) indicates significant group difference in the survival of larvae in between 0.50 and 1.00 ml and 0.75 and 1.00 ml *A. vera* essential oil in case of

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

	Aloe vera essential oil applied (ml)				
Stage of treatment (larval instar)	Control (X_1)	0.25 (X_2)	0.50 (X_3)	0.75 (X_4)	1.00 (X_5)
Single	23.186±0.216	22.980±0.214	22.863±0.480	22.561±0.472	23.502±0.399
(5th)	(100)	(99.11)	(98.61)	(97.30)	(101.36)
Double	23.186±0.216	22.756±0.288	22.521±0.466	22.232±0.137	24.135±0.133
(4th -5th)	(100)	(98.15)	(97.13)	(95.89)	(104.09)
Triple	23.186±0.216	22.511±0.399	22.151±0.201	21.436±0.313	25.035±0.330
(3rd -5th)	(100)	(97.09)	(95.54)	(92.45)	(107.97)

- F_1 = 14.5406 (n_1=4, n_2=38), P < 0.01; F_2 = 0.2240 (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 larval 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 larval duration of *Bombyx mori*.

	Stage of treatment		
Mean difference in between groups	Single	Double	Triple
$X_1 \sim X_2$	0.206	0.430	0.675
$X_1 \sim X_3$	0.323	0.665	1.035
$X_1 \sim X_4$	0.625	0.954	1.750
$X_1 \sim X_5$	0.316	0.949	1.849
$X_2 \sim X_3$	0.117	0.235	0.360
$X_2 \sim X_4$	0.419	0.524	1.075
$X_2 \sim X_5$	0.522	1.379	*2.524
$X_3 \sim X_4$	0.302	0.289	0.715
$X_3 \sim X_5$	0.639	1.614	*2.884
$X_4 \sim X_5$	0.941	*1.903	*3.599

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

$= \sqrt[5.05]{\frac{0.4137}{3}}$

$= 1.875$

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 larval 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* larvae.

	Aloe vera essential oil applied (ml)				
Stage of treatment (larval instar)	Control (X_1)	0.25 (X_2)	0.50 (X_3)	0.75 (X_4)	1.00 (X_5)
Single	76.248±0.896	78.343±0.578	79.147±0.477	81.732±0.581	72.685±0.570
(5^{th})	(100)	(102.75)	(103.80)	(107.19)	(95.33)
Double	76.248±0.896	83.076±0.320	84.582±0.297	90.884±0.954	68.551±0.393
(4^{th} -5^{th})	(100)	(108.95)	(110.93)	(119.20)	(89.91)
Triple	76.248±0.896	88.848±0.616	90.623±0.628	96.427±0.681	56.858±0.942
(3^{rd} -5^{th})	(100)	(116.53)	(118.85)	(126.46)	(74.57)

- $F_1 = 28.8255$ (n_1=4, n_2=38), $P < 0.01$; $F_2 = 2.6469$ (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 survival per cent of larvae 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%.

double treatment of larvae. In the triple treatment of larvae, significant group difference in the survival of larvae was noticed in between 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.

DISCUSSION

The rich nutrients of mulberry leaves enhanced its nutritional status causing reduction in the larval duration [23]. The variation in the larval duration of silkworm has been reported by a number of workers [24, 25]. Variation in the photoperiod affected the larval duration [26], while the silkworm larvae were exposed to 24 hours light a day, the larval span was prolonged [27]. It is well known that an ideal race is one which has a shorter duration thus causing low consumption of leaf [28]. The rearing condition was also reported to be effective in deciding the larval span and growth [29], seasonal variation effected larval duration [30]. Refrigeration of egg influenced the larval duration [11]. The higher magnetic field strength [31], reduction in the larval duration under 20 minute exposure at 3500 gauss [32] and cocoon magnetization

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

	Stage of treatment		
Mean difference in between groups	Single	Double	Triple
$X_1 \sim X_2$	2.095	6.828	12.600
$X_1 \sim X_3$	2.899	8.334	14.375
$X_1 \sim X_4$	5.484	14.636	*20.179
$X_1 \sim X_5$	3.563	7.697	*19.390
$X_2 \sim X_3$	0.804	1.506	1.775
$X_2 \sim X_4$	3.389	7.808	7.579
$X_2 \sim X_5$	5.658	14.525	*31.990
$X_3 \sim X_4$	2.585	6.302	5.804
$X_3 \sim X_5$	6.462	*16.031	*33.765
$X_4 \sim X_5$	9.047	*22.333	*39.569

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

$= {}_{5.05}\sqrt{\frac{26.345}{3}}$

$= 14.965$

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 larvae in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* essential oil treatment, respectively

also influences the larval duration [13]. Application of manta [33], juvenile hormone [34] and topical application of methoprene (Juvenile Hormone Analogue) prolonged larval period of *B. mori* [35]. The phytoecdysteriod is recognized as one of the most important components in the silkworm rearing [36], while synthetic juvenoid (R394) treatment caused prolongation the larval duration [37, 38] and the minimum larval duration was noticed in case of triple treatment by 60% ecdysone concentration [14]. Ascorbic acid affects the larval duration [39], antibiotics affect larval duration [40, 41] and treatment of *B. mori* eggs with HCL influenced larval duration [42]. Fed mulberry leaves treated with *Nux vomica* [43], folic acid administration [44] and herbal tonic influenced larval duration of *B. mori* L. [20]. Larvae fed on soyabean and mushroom diet gave the shortest larval duration of *B. mori* larvae [45]. Nuclear polyhedrosis virus (NPV) influenced larval duration [46]. Exposure of garlic volatile [47] and ultrasound [48] affected the larval duration of *B. mori*. Mulberry varieties S-1635 influenced larval duration [49].

The exposure to high temperature reduced the survival [50], while temperature between 23.9 to 25.8 °C along with 90.9% relative humidity best for survival of *B. mori* larvae [51]. Relative humidity [9] and ecological factors [10] influenced survival of *B. mori* larvae. Seasonal variation [30] and mulberry varieties and season also influenced survival of *B. mori* larvae [24]. Genome of silkworm regulates the survival of larvae [52]. Magnetization influenced survival of *B. mori* larvae [32] and cocoon magnetization influenced survival of *B. mori* larvae [13]. The topical application of methoprene juvenile hormone analogue good for survivability of larvae [35], 95% larval survival was observed after treatment R-394 in *B. mori* [53] and 20-Hydroxyecdysone influenced the 18.20% survival percent of larvae [14]. Antibiotics influenced survival of *B. mori* larvae [40], ascorbic acid enhances survival rate of larvae [54]. Vitamins complex treatment caused significant increase in survival of larvae [55] and mulberry leaves sprayed with linseed oil, hemp oil and milk influenced the survival of larvae [16]. Medicinal plant extract *Phyllanthus niruri* reduced (11%) larval mortality [56]. *A. vera* gel affects the growth and survival performance of *Rainbow trout* [57]. Survival of larvae increased when mulberry leaves treated with bovine milk [58], larval fed on soybean and mushroom diet affected the survival of *B. mori* larvae [45] and garlic volatile exposure duration caused increase in survival of *B. mori* larvae [59].

On the basis of present observation and above information it may be concluded that larval duration decreased with the increase in the number of treatment with different amount of *A. vera* essential oil but with the increasing number of larval treatment from one to three

times, the survival of larvae increased in case of 0.25, 0.50 and 0.75 ml *A. vera* essential oil. 1.00 ml *A. vera* essential oil treatment caused notable decline in the survival of larvae with increase in the number of larval treatment from single to triple. *A. vera* essential oil simulates silkworm larvae for tolerance to toxin and viral infection, accelerates growth and development in silkworm.

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