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Effect of *Aloe vera* oil influences the fecundity and hatchability of multivoltine mulberry silkworm (*Bombyx mori* Linn.)

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ABSTRACT

The application of *Aloe vera* oil on the *Bombyx mori* has been proved to be a significance in the sericulture industry. The experiments were conducted with amount of *A. vera* oil viz, 0.25, 0.50, 0.75, and 1.0 ml as single, double and triple treatment with respect to the treatment of 3rd, 4th and 5th instar *B. mori* larvae. The maximum level of fecundity (409.66±3.31 eggs) and hatchability (96.75±1.22 %) observed in case of triple treatment with 0.75 ml amount of *A. vera* oil. Minimum fecundity (270.00±1.86 eggs) and hatchability (75.00±1.60 %) was noticed to be in case of triple treatment with 1.0 ml amount of *A. vera* oil. If *A. vera* oil applied tactfully, may boost up the formation of good cocoons at commercial scale.

Keywords: *A. vera* oil, *Bombyx mori*, Reproductive ability.

Introduction

The mulberry silkworm, *Bombyx mori L.* is essentially monophagous insect and survive solely on mulberry leaves which play an important role in the nutrition of the silkworms. Rearing of silkworm larvae at lower levels of RH resulted in lower fecundity, hatchability (Hussain et al. 2011). *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. The supplementation and fortification of mulberry leaves is recent technique in sericulture research (Murugan et al. 1998). Treating eggs were characterized by pure white egg colour instead of the pale yellow of normal eggs shortly after oviposition (Fujikawa et al. 1933a). *A. vera* is native of the mediterranean region of southern Europe and North Africa. 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, Upadhyay and Tripathi, 2005), cocoon refrigeration (Upadhyay, et al. 2009), cocoon magnetization (Upadhyay and Prasad, 2010), 20-hydroxyecdysone hormone (Prasad and Upadhyay, 2012) and phytoecdysteroid hormone (Upadhyay and Pandey, 2012) Srivastava and Upadhyay, 2013) 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). Man 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 rearers (Nair and Kumar, 2004). 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 (Abdelgaleil et al. 2009). *A. vera* herbal tonic 'Iogen' (Balamurugan and Isaiarasu, 2007) alloe (Manimutha and Isaiarasu, 2010) and Aloe tonic treated mulberry leaves (Deshmukh and Khyade, 2013) influence the cocoons, pupal and growth parameters of B. mori. Plant produced insect moulting hormones phytoecdysteroids (PES), act either as feeding deterrents or against that induce developmental disruption (Schmulz et al. 2002). The plant like *Achyranthes aspera* (Lat jeera) and *Cassia tora* (choti chakwar) have been identified as source of phytoecdysterod (Lafont and Horn, 2004). Keeping this in view, it is hypothesized that *B. mori* larvae treated with A. vera oil may cause certain beneficial effects on the reproductive ability of *B. Mori.*

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 (Krishnaswami et al. 1973) 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

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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. 3^{rd}, 4^{th} and 5^{th} 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 3^{rd}, 4^{th} and 5^{th} 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.

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 4^{th} instar larvae. In the first treatment, 100 larvae of fourth instar were treated just before two days of 4^{th} 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 5^{th} instar larvae i.e. just before two days of spinning.

Triple treatment of larvae-: For triple treatment, the 3^{rd} instar larvae just before 3^{rd} moulting were separated from BOD incubator. In the first treatment, 100 larvae of 3^{rd} 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 4^{th} moulting i.e. at the final stage of 4^{th} instar larvae and transferred in BOD of spinning. Thus, in the triple treatment 3^{rd}, 4^{th}, and 5^{th} 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.

Fecundity-: For determining the fecundity, 15 layings (three batches of five layings in each batch) were taken for each replicate. Thus, average of five layings was taken as representative number of eggs laid by a female moth in case of each set of experiment. Three replicates of each experiment were made.

Hatching per Cent-: At head pigmentation stage, the eggs under incubation were black boxed and exposed to diffused light on the date of hatching. After complete hatching (third day from the beginning of larval hatching) the disease free layings were counted to collect the data in respect to the total number of eggs laid per female moth, number of unfertilized eggs and number of hatched eggs per laying. The average hatching of 10 layings were taken as representative hatchability percentage per layings in case of each batch of the study. Thirty layings (three batches of the 10 layings in each batch) were counted for each replicate. Three replicates of each experiment were made. The hatchability was calculated as follows-

$$\text{Hatchability (\%)} = \frac{\text{No. of eggs hatched}}{\text{No. of eggs fertilized}} \text{ X } 100$$

Result

Fecundity-: The data presented in (table 1a) clearly indicates that variation in the amount of *Aloe vera* oil and the number of treatment influenced the fecundity of adult female moth. With the increasing number of treatment from one to three times. The fecundity increased in case of 0.25, 0.5, 0.75 ml amount of *Aloe vera* oil while in case of 1.0 ml amount of *Aloe vera* oil, the fecundity decreased in single, double and triple treatment of *Bombyx mori* larvae and reached to minimum level in case of triple treatment with 1.0 ml amount of *Aloe vera* oil. 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, 0.5, 0.75 ml amount of *Aloe vera* oil. The maximum fecundity of adult moth was noticed to be 409.66±3.31 eggs in the triple treatment with 0.75 ml amount of *Aloe vera* oil. The minimum fecundity was recorded 270.00±1.86 eggs 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 fecundity of adult female moth. (table 1a) while the Post hoc test (table 1b) indicates no group difference was found in case of single treatment. In case of double treatment significant group difference was found in the fecundity in between 0.25 and 1.0 ml, 0.5 and 1.0 ml, 0.75 and 1.0 ml amount of *Aloe vera* oil. In triple treatment significant group difference was found in the fecundity in between control

and and 0.5 ml, control and 0.75 ml, 0.25 and 1.0 ml, 0.5 and 1.0 ml, 0.75 and 1.0 ml.

Hatching per cent: The data given in (table 2a) clearly indicates that variation in the amount of *Aloe vera* oil and the number of treatment influenced the hatching per cent of adult female moth eggs. With the increasing number of treatment from one to three times, the hatching per cent increased in case of 0.25, 0.5, 0.75 ml amount of *Aloe vera* oil while in case of 1.0 ml amount of *Aloe vera* oil, the hatching per cent decreased in single, double and triple treatment of *Bombyx mori* larvae. The trend of increase in the hatching per cent 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 *Aloe vera* oil treatment. The maximum hatching per cent of adult female moth was noticed to be 96.75±1.22 % in the triple treatment with 0.75 ml amount of *Aloe vera* oil treatment. The minimum hatching per cent was recorded 75.00±1.60 % in case of triple treatment by 1.0 ml amount of *Aloe vera* oil.

Two way ANOVA indicates that the variation in the amount of *Aloe vera* oil treatment significantly ($P_1 < 0.05$) influenced the hatching per cent of adult female moth eggs. (table 2a) while the Post hoc test (table 2b) indicates significant group difference in the hatching percent of eggs in between 0.75 ml and 1.0 ml amount of *Aloe vera* oil in case of double treatment. In triple treatment significant group difference in the fecundity was observed in between control and 0.75 ml, control and 1.0 ml, 0.25 and 1.0 ml, 0.5 and 1.0 ml, 0.75 and 1.0 ml amount of *Aloe vera* oil treatment. No group difference was found in case of single treatment.

Discussion

Fecundity: The fecundity and fertility are two major components of silkworm seed production. The relationship between the fecundity and adult density of *Philosamia ricini* was inversely proportional to the number of moth pairs in the container (Srivastava and Mishra, 1985). It was estimated that the gel of *Aloe-vera* (L) contains nearly 1.7% Protein (Luta, et al., 2009). The variation in the number of eggs occurred due to fluctuation of temperature and humidity and interaction with silkworm lines. The egg laying capacity of *Bombyx mori L.* has been noticed to be influenced by the genotype of silkworm line and rearing temperature. The fecundity and fertility are two major components of silk worm seed production. Fecundity of the moths emerged from the pupae of refrigerated eggs (Pandey and Upadhyay, 2001) Whereas rearing of silkworm larvae at lower levels of Rh resulted in lower fecundity, hatchability (Hussain, et al., 2011). The secrets growth and development of *B. mori* (L) lies in wealth nutrition (kanafi, et al., 2007). The present work is a preliminary study on the use of *A. vera* oil for the determination of the reproductive ability of must commercially important insects. Therefore, future more comprehensive works are very much solicited in this line with highest doses on this species is required. The present study was only an attempt to assess the influence of *A. vera*, which is used extensively as a food additive by virtue of this nutritive and medicinal properties. Medicinal herbal formulations should be screened for growth promoting activity in the silkworm *B. mori* (L). In female insects, the steroid hormone 20-hydroxyecdysone (20E) plays a major role in activating vitellogenesis, a process required for egg development (Pon deviile, et al., 2008). The PES are not hypersensitive, androgenic, oestrogenic or anti oestrogenic and do not induce vitilisation (Dinnan, et al., 2009). The eggs laying efficiency of silk moths, obtained from the heavy pupae, was notably highly significant positive correlation of pupal weight with the fecundity has been noticed in some other sericogenous moth viz., *Philosamia ricini* (Singh and Prasad, 1987) and S*amia Cynthia ricini* (Nagalakashmanna et al. 1988). The heavy fecundity was noticed in the moths, obtained from *B. mori* larvae feeding on ascorbic acid treated mulberry leaves (Rahman, et al., 1990). The exposure of gamma radiation of *B. mori* eggs caused an increase in the fecundity (Adbel Salam and Mahmoud, 1995). The insect reproductive activity is controlled by juvenile hormone (Doane, 1973) and ecdysone (Parlak, et al., 1992). The relationship between the fecundity and adult density of *Philosamia ricini* was inversely proportional to the number of moth pairs in the container (Srivastava and Mishra, 1985). The fecundity of *B. mori* varies basically due to variation in the race of silkworm (Thomas and Dale, 1997). The vitellogenic female protein necessary for the growth of oocytes is already abundant in the haemolymph of *B. mori* pupae before the maximum secretion of ecdysone before moulting from the prothoracic glands (Kawaguchi and Doira, 1973).

Hatching per cent: The occurrence of unfertilized eggs was more common in summer as compared to other seasons (Biran, et al., 2009). The increasing duration of refrigeration of *B. mori* eggs caused notable decline in the hatching percent (Venkataramn and Prabhakar, 1980; Pandey and Upadhyay, 2001). Generally mutants are inferior to the normal in several characters such as fecundity and hatching (Rajanna, et al., 2012) variation in the 20-hydroxyecdysone concentration significantly ($P < 0.05$) influenced the reproductive potential of *B. mori* in terms of fecundity and hatchability of eggs (Prasad and Upadhyay, 2012). The hatchability of eggs increased with the increasing *A. vera* oil doses from 0.25 ml to 0.75 ml while it declined with 1.0 ml at the same treatment stages. Thus, it may be concluded that with the increasing *A. vera* oil doses up to 0.75 ml. The hatching percent of eggs increases due to activation of enzyme activities in embryonic stage. The variation in the *A. vera* oil treatement and the number of larval treatment caused notable influence on the hatchability of eggs. The survival and development of insects are at the mercy of nature and developmental activities are restricted in accordance with the prevailing ecological conditions and to a certain extent to their genetic built up (Andrewartha and Birch, 1954; Roberston, 1957 and Krishnaswamy, et al., 1973). The multiple and signle mating made no significant difference in the percent hatching of *B. mori* eggs (Singh et al. 1994). Whereas the treatment of *B. mori* eggs with HCL (6N) caused higher hatchability of eggs (Tiburcio, 1985 and Hurkadli, 1998). The magnetization of eggs significantly increased the weight of silk gland of *B. mori* (Upadhyay and Tripathi, 2006). The refrigeration of eggs of early stage has adverse effect on the hatchability of insect eggs (Govindan, et al., 1980; Krishaba and Henneberry, 1966).

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It is concluded that treatment mulberry leaves with different amount of *Aloe vera* oil and feeding to the 3rd ,4th and 5th instar larvae of silkworm, *Bombyx mori* (L) resulted in elicitation better response of reproductive ability (Fecundity, Hatchability) in 0.75 ml. The higher amount of *Aloe vera* oil (1.0 ml) may cause stress response causing decrease in the enzyme activities as a result of reproductive ability decreased.

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APPENDIX

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

Stage of treatment (larval instar)	*Aloe vera* oil applied (ml)					F_1-ratio
	Control (X_1)	0.25 (X_2)	0.50 (X_3)	0.75 (X_4)	1.00 (X_5)	$n_1 = 4$
Single (5^{th})	325.00±1.86	334.66±3.61	344.66±0.55	365.33±1.91	310.33±2.16	
Double (4^{th} -5^{th})	325.00±1.86	353.66±4.42	365.00±3.59	380.00±1.71	285.00±2.23	**12.038***
Triple (3^{rd} -5^{th})	325.00±1.86	375.33±2.17	390.00±2.44	409.66±3.31	270.00±1.86	

F_2 –ratio = 1.180** $n_2 = 2$

*$P_1 < 0.01$

**Non 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 fecundity in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.

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

Mean difference in between groups	Stage of treatment		
	Single	Double	Triple
$X_1 \sim X_2$	9.66	28.66	50.33
$X_1 \sim X_3$	19.66	40.00	*65.00
$X_1 \sim X_4$	40.33	55.00	*84.66
$X_1 \sim X_5$	14.67	40.00	55.00
$X_2 \sim X_3$	10.00	11.34	14.67
$X_2 \sim X_4$	30.67	26.34	34.33
$X_2 \sim X_5$	24.33	*68.66	*105.33
$X_3 \sim X_4$	20.67	15.00	19.66
$X_3 \sim X_5$	34.33	*80.00	*120.00
$X_4 \sim X_5$	55.00	*95.00	*139.66

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

$$= 5.05\sqrt{\frac{358.130}{3}}$$

$$= 55.176$$

MS = 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 fecundity 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 hatching per cent (%) of *Bombyx mori.*

Stage of treatment (larval instar)	*Aloe vera* oil applied (ml)					F_1-ratio
	Control (X_1)	0.25 (X_2)	0.50 (X_3)	0.75 (X_4)	1.00 (X_5)	$n_1 = 4$
Single (5^{th})	85.00±1.75	86.75±1.23	88.75±1.79	91.25±1.39	84.50±1.72	
Double (4^{th} -5^{th})	85.00±1.75	88.50±1.89	91.75±2.00	93.75±1.59	83.25±1.75	7.447*
Triple (3^{rd} -5^{th})	85.00±1.75	90.75±1.34	94.00±1.64	96.75±1.22	75.00±1.60	

F_2 –ratio = 0.198** $n_2 = 2$

*$P_1 < 0.05$

**Non 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 hatching per cent (%) 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 hatching per cent (%) of *Bombyx mori*.

Mean difference in between groups	Stage of treatment		
	Single	Double	Triple
$X_1 \sim X_2$	0.60	1.25	2.20
$X_1 \sim X_3$	1.30	2.64	3.54
$X_1 \sim X_4$	2.41	3.34	*4.59
$X_1 \sim X_5$	0.33	0.77	*3.74
$X_2 \sim X_3$	0.70	1.39	1.34
$X_2 \sim X_4$	1.81	2.09	2.39
$X_2 \sim X_5$	0.93	2.02	*5.94
$X_3 \sim X_4$	1.11	0.80	1.05
$X_3 \sim X_5$	1.73	3.41	*7.28
$X_4 \sim X_5$	2.74	*4.11	*8.33

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

$$= 5.05\sqrt{\frac{1.531}{3}}$$

$$= 3.606$$

MS = 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 hatching per cent (%) in control, 0.25, 0.50, 0.75 and 1.00 ml *Aloe vera* oil respectively.

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