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Original Research Article

Influence of Oil (*Aloe-vera*) on the Glucose Content at the Initial and Final Stage in the Haemolymph of Multivoltine Mulberry Silkworm Pupae (*Bombyx mori* LINN).

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A B S T R A C T

The significance of *Aloe vera* oil on *Bombyx mori* cocoon has been proved to be of biotechnological significance in the sericulture industry. Variation in the oil of *Aloe vera* significantly influenced the glucose content at the initial and final stage of haemolymph of *Bombyx mori* pupae. 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 3, 4 and 5 instar *B. mori* larvae. A control set was also arranged for each larval instar. The maximum level of glucose content at the initial stage of haemolymph (13.260±0.039 µg/mg) was noticed in case of triple treatment by 0.75 ml. amount of *Aloe-vera* oil. The minimum glucose content at the final stage of haemolymph (9.667±0.015 µg/mg) was recorded in case of triple treatment by 0.1 ml. amount of *Aloe-vera* oil. In conclusion, it may be suggested that, if applied tactfully, may be useful for boosting up the sericulture industry as well as the economical purpose of silkworm rearing.

Keywords

Haemolymph, 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.

Glucose

The glucose content in experimental tissues of different developmental stages, obtained in continuation of the earlier experiments was estimated.

The glucose level was estimated according to 'Kemp and Mayer's method (1954). For determining the glucose content, the tissue viz: 31 ml. Haemolymph, 25 mg. Fat body and 30 mg. Silk gland were deproteinized separately with 5% TCA, containing 0.1% silver sulphate. The content, thus obtained, was centrifuged at 10,000 rpm. for 10 minutes. Further, in 0.5 ml of deproteinized supernatant, 4.5 ml of H2SO4 was added thoroughly by shaking. Now, content was boiled in water bath for 6 minutes and the mixture was cooled at room temperature. The pink colour obtained, was observed, at 520 nm by the spectrophotometer. The blank consisted of 0.5 ml of TCA containing 0.1% silver sulphate and 4.5 ml. of Molar H2SO4 which was given the same treatment as that of the experimental samples. Standard curve was prepared with different concentrations of pure glucose solution. The glucose content in different tissues was expressed as µg/mg of tissue. The experiment was conducted for different stages of the development. Six replicates of each experiment were made.

Results and Discussion

Glucose content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the initial stage of spinning

The data presented in table 1a clearly indicates that variation in the amount of *Aloe vera* oil and the number of larval treatment influenced the glucose content in the haemolymph of larvae at the initial stage of spinning. With the increasing number of larval treatment from one to two times, the glucose content in the haemolymph of larvae at the initial stage of spinning increased in case of 0.25, 0.50 and 0.75ml of *Aloe vera* oil treatment but the triple treatment of larvae caused notable decline in the glucose content in the haemolymph of larvae at the initial stage of spinning in all the above cases. 1.00ml *Aloe vera* oil treatment caused notable decline in the glucose content in the haemolymph of larvae at the initial stage of spinning with increase in the number of larval treatment from single to triple. The trend of increase in the glucose content in the haemolymph of larvae at the initial stage of spinning with the increasing number of larval treatment has been recorded to be almost of similar trend in case of 0.25, 0.50 and 0.75ml *Aloe vera* oil treatment. The maximum glucose content in the haemolymph of larvae at the initial stage of spinning was noticed to be 6.20±0.782 µg/mg in case of triple treatment of larvae by 0.75ml of *Aloe vera* oil and the minimum glucose of 5.35±0.843 µg/mg was recorded in case of triple treatment of larvae by 1.00ml amount of *Aloe vera* oil.

Two way ANOVA indicates that the number of larval treatment significantly (p_2<0.05) influenced the glucose content in the haemolymph of larvae at the initial stage of spinning. The post-hoc test table 1b indicates significant group difference in the glucose content in the haemolymph of larvae at the initial stage of spinning. There was any no significant group difference was found in single, double and triple treatment.

Glucose content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the final stage of spinning

The data presented in table 1a clearly indicates that variation in the amount of *Aloe vera* oil and the number of larval treatment influenced the glucose content in the

haemolymph of larvae at the final stage of spinning. With the increasing number of larval treatment from one to two times, the glucose content in the haemolymph of larvae at the final stage of spinning increased in case of 0.25, 0.50 and 0.75ml of *Aloe vera* oil treatment but the triple treatment of larvae caused notable decline in the glucose content in the haemolymph of larvae at the final stage of spinning in all the above cases. 1.00ml *Aloe vera* oil treatment caused notable decline in the glucose content in the haemolymph of larvae at the final stage of spinning with increase in the number of larval treatment from single to triple. The trend of increase in the glucose content in the haemolymph of larvae at the final stage of spinning with the increasing number of larval treatment has been recorded to be almost of similar trend in case of 0.25, 0.50 and 0.75ml *Aloe vera* oil treatment. The maximum glucose content in the haemolymph of larvae at the final stage of spinning was noticed to be 7.53±1.766 µg/mg in case of triple treatment of larvae by 0.75ml amount of *Aloe vera* oil and the minimum glucose of 5.54±1.480 µg/mg was recorded in case of triple treatment of larvae by 1.00ml amount of *Aloe vera* oil.

Two way ANOVA indicates that the number of larval treatment significantly (p_2<0.05) influenced the glucose content in the haemolymph of larvae at the final stage of spinning. The post-hoc test table 2b indicates significant group difference in the glucose content in the haemolymph of larvae at the final stage of spinning, in the double treatment, in between control and 0.75ml, 0.75 and 1.00ml. In triple treatment, in between control and 0.75ml, 0.5 and 1.00ml, 0.75 and 1.00ml. In single treatment, significant group difference was not found.

Table.1a Effect of essential oil (*Aloe vera* oil) on the glucose content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the initial stage of spinning

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 (5^{th})	5.48±1.037	5.54±0.973	5.57±0.850	5.70±1.145	5.67±1.032
Double (4^{th} -5^{th})	5.48±1.037	5.62±0.854	5.68±1.154	5.96±1.038	5.46±0.876
Triple (3^{rd} – 4^{th}- 5^{th})	5.48±1.037	5.73±0.930	5.86±1.217	6.20±0.782	5.35±0.843

F_1 = 1.1956 (n_1=4, n_2=38), not significant, F_2 =0.2895 (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 glucose content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the initial stage of spinning in control, 0.25, 0.50, 0.75 and 1.00ml treatment of *Aloe vera* oil respectively.

Table.1b Post-hoc test showing effect of essential oil (*Aloe vera* oil) on the glucose content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the initial stage of spinning

Mean difference in between groups	**Stage of treatment**		
	Single	**Double**	**Triple**
$X_1 \sim X_2$	0.006	0.014	0.250
$X_1 \sim X_3$	0.009	0.200	0.380
$X_1 \sim X_4$	0.22	0.48	0.72
$X_1 \sim X_5$	0.190	0.020	0.130
$X_2 \sim X_3$	0.030	0.140	0.130
$X_2 \sim X_4$	0.060	0.200	0.230
$X_2 \sim X_5$	0.130	0.220	0.380
$X_3 \sim X_4$	0.090	0.020	0.100
$X_3 \sim X_5$	0.110	0.220	0.570
$X_4 \sim X_5$	0.190	0.240	0.610

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

$= 6.10\sqrt{\frac{0.2313}{6}}$

$= 1.1968$

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 the mean values of glucose content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the initial stage of spinning in control, 0.25, 0.50, 0.75 and 1.00 ml treatment of *Aloe vera* oil respectively.

Table.2a Effect of essential oil (*Aloe vera* oil) on the glucose content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the final stage of spinning

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})	5.63±1.128	5.83±1.078	6.03±0.914	6.75±1.049	6.18±0.779
Double (4^{th} -5^{th})	5.63±1.128	6.10±0.943	6.44±0.824	7.24±1.199	5.63±1.157
Triple (3^{rd}-4^{th}-5^{th})	5.63±1.128	6.19±0.859	6.94±1.117	7.53±0.952	5.54±0.885

F_1 = 10.9721 (n_1=4, n_2=38), P_1 < 0.01; F_2 = 0.9473 (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 glucose content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the final stage of spinning in control, 0.25, 0.50, 0.75 and 1.00 ml treatment of *Aloe vera* oil respectively.

Table.2b Post-hoc test showing effect of essential oil (*Aloe vera* oil) on the glucose content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the final stage of spinning

Mean difference in between groups	**Stage of treatment**		
	Single	**Double**	**Triple**
$X_1 \sim X_2$	0.20	0.47	0.56
$X_1 \sim X_3$	0.40	0.81	1.31
$X_1 \sim X_4$	1.12	*1.61	*1.90
$X_1 \sim X_5$	0.55	0	0
$X_2 \sim X_3$	0.20	0.34	0.75
$X_2 \sim X_4$	0.92	1.14	0.59
$X_2 \sim X_5$	0.35	0.47	0.64
$X_3 \sim X_4$	0.72	0.80	0.59
$X_3 \sim X_5$	0.15	0.81	*1.40
$X_4 \sim X_5$	0.57	*1.61	*1.99

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

= $6.10\sqrt{\frac{0.3163}{6}}$

= 1.4006

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 the mean values of glucose content (µg/mg) in the haemolymph of *Bombyx mori* larvae at the final stage of spinning in control, 0.25, 0.50, 0.75 and 1.00 ml treatment of *Aloe vera* oil respectively.

Total glucose content

The total glucose content in the silk gland has been noticed to be of increasing trend with the increasing number of larval treatment by *Aloe vera* oil from one to three times in case of 0.25, 0.50 and 0.75 ml *Aloe vera* oil while with 1.00 ml, the glucose content was decreased. The significant variation in the level of glucose and

trehalose in different tissues like silk gland, fat body and haemolymph characterized the thermal adaptation of eri silkworm (Singh, 1980). Adaptation of *Galleria mellonella* larvae at 2^{o}c resulted in the accumulation of glucose accompanied by simultaneous decrease in the trehalose of the whole larval homogenate (Lenartouiez and Niemierka, 1968).

The level of glucose in the fat body of *Bombyx mori* larvae was influenced due to variation in the number of larval treatment and different amount of *Aloe vera* oil. With the increasing number of larval treatment by *Aloe vera* oil from one to three times, the glucose content was increased in case of 0.25, 0.50 and 0.75 ml of *Aloe vera* oil while in 1.00 ml of *Aloe vera* oil, the glucose content in the fat body increased in single treatment while further increase in the larval treatment caused decline in glucose level which reached to the lowest level in case of triple treatment. The maximum glucose level was noticed in case of triple treatment of 0.75 ml. of *Aloe vera* oil. A number of reactions of intermediary metabolism related to carbohydrate, protein, fat and nucleic acid have been demonstrated to occur in insect fat body (Kilby, 1963; and Wyatt, 1974). The insect fat body has been recognized as the main site of trehalose biosynthesis from glucose which may be readily supplied through breakdown of the glycogen reseeves maintained in the tissues (Candy and Kilby, 1959 and 1961; and Wyatt, 1974). The synthesis and catabolism of trehalose is a process which has some importance in the economy of insects and this process is intimately linked to the metabolism of glucose and glycogen (Prosser, 1973). An inverse relationship was found in between the glycogen and trehalose in the fat body of silkworm and sufficient amount of glucose in 5th instar larvae may be made available through glycogenolysis and also through trehalose hydrolysis during the 4th ecdysis in *Philosamia ricini* (Singh and Singh, 1978). The total carbohydrate content in the *Bombyx mori* larvae treated with JHA394 is largely dose dependent which was lowest on higher amount of *Aloe vera* oil (Nair *et al.*, 2007). Magnetic field influences the glucose content in the fat body of *B. mori* larvae (Prasad and Upadhyay, 2011). Cocoon magnetization influences the glucose content in fat body and haemolymph of *Bombyx mori* pupae (Prasad and Upadhyay, 2012). Phytoecdysteriod treatment influenced glucose content in the silk gland, fat body and haemolymph of multivoltine *Bombyx mori* at initial Stage of Spinning (Pandey and Upadhyay, 2013).

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