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Influence of Different Nutritional Sources on Various Developmental and Biological Aspects of Silkworm, *Bombyx mori* L.

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Abstract: *Bombyx mori* L., larvae were reared on Mulberry, *Morus laevigata* L., treated with macro and micro nutrients in different combinations. Out of a lot of 13 characters of Silkworm larvae, all of them were found to have been significantly affected by the various nutritional packages. The magnitude of changes in an over all PC-1 proportion 1000 pound to be 0.950 for the developmental aspects [Table 4.1 (E)] and 0.953 for the data on the biological aspects of the silkworm [Table 4.2 (E)]. Statistically, on over all basis, the upsets introduced by the nutritional sources for different character sets were observed to be 98.88% [Table 5.1 (E)] and 98.84% [Table 5.2 (E)] with reference to the developmental aspects and bionomical characters, respectively. On comparative basis, however, an R^2-value of 0.9294 for the larval body weight [Table 5.1 (V_3 / D)] and of 0.8681 for the female life span [Table 5.2 (V_{10} / D)], shall indicate that these characters have been influenced to the maximum. Thus, a nutritional package in T_9 comprising the optimum doses of 9 different nutrients viz., N (0.2%) + P (0.1%) + K (0.3%) + Ca (0.1%) + Mg (0.1%) + Zn (0.15%) + Cu (0.05%) as well as Fe (0.1%) and Mn (0.15%) was found to have been the best of the tested package-lots.

Key words: Nutritional sources · developmental aspects · biological aspects · mulberry silkworm · *Bombyx mori*

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L., (Bombycidae, Lepidoptera) is reckoned to be one of the commercial insects. The silkworm larvae have a high medicinal value and are usually used to reduced blood pressure, diabetes, nerve disorders and heart problems. In addition to the uses of its larvae for silk production, its pupae are being employed to extract vitamins A, B12, E and K, where as the male moths are utilized for making medicinal vines and the excreta forms an important part of the fish and poultry-feed [1].

The studies under taken by Patil *et al.* [2] on the eri-silkworm, raised on mulberry leaves, enriched with 1, 5, 10, 15 and 20% solutions of the leaf-extract of *Parthenium hysterophorus*, the leaf-extract, stimulated the silkworm larvae not only to consume / utilize more food, but also to grow up into a bigger size and produce bigger cocoons, with significant improved economic traits. Similarly in the same year Vishwanath *et al.* [3] sorted out the effect of mineral supplementation of mulberry leaves, on the development of *B. mori* L., larvae. It was found by them, that this practice had a positive effect not only on the larval body weight, but also on the larval body length, in contrast to a negative effect on the larval duration. A bit later, during 1998, Bhoopathy and Gunasegar, raised a hybrid *B. mori* silkworm (LR×NB 22) on the mulberry leaves, supplemented with four different concentrations of potassium dichromate (K_2 Cr_2 O_7). The experimental Record, showed a conspicuous increase not only in the larval weight and size of the silk gland but also in the pupal weight, cocoon weight, shell weight as well as in the filament length, as against a decrease of the larval duration. Shankar *et al.* [4] tried to record the impact of nitrogen from different sources, on the grain-age parameters of silkworm, *Bombyx mori* L. and concluded that there was an increase in its fecundity and hatching percentage, but a decrease in the number of dead eggs, when feed on the leaves produced by applying nitrogen in the form of calcium-ammonium nitrate. Likewise Jadhav and his colleagues, during 2000, in a field experiment, investigated the effect of the application of organic and inorganic manures + their combinations on the leaf quality of mulberry [5]. They reported that by the application of a combination of organic and inorganic fertilizers, the carbohydrate and crude protein percentage

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Table 1: Total mineral matter contents (g) employed in each treatment for 3 applications

Treatments	
T_0	Simple Mulberry leaves
T_1	N.2%
T_2	N.2%+P.1%
T_3	N.2%+P.1%+K.3%
T_4	N.2%+P.1%+K.3%+Ca.1%
T_5	N.2%+P.1%+K.3%+Ca.1%+Mg.1
T_6	N.2%+P.1%+K.3%+Ca.1%+Mg.1+Zn.15%
T_7	N.2%+P.1%+K.3%+Ca.1%+Mg.1+Zn.15%+Cu.05%
T_8	N.2%+P.1%+K.3%+Ca.1%+Mg.1+Zn.15%+Cu.05%+Fe.1%
T_9	N.2%+P.1%+K.3%+Ca.1%+Mg.1+Zn.15%+Cu.05%+Fe.1%+Mn.15%

of the mulberry leaves increased, which, in turn, significantly increased the larval body weight, silk gland weight and ultimately the cocoon yield etc.

MATERIALS AND METHODS

These investigations were initiated, in the Entomological Research Laboratories of the New Insectary Building, University of Agriculture, Faisalabad (Punjab : Pakistan) with a combination of *Bombyx mori* L., the mulberry silkworm, larvae and the leaves of *Morus laevigata* L., treated with 9 different nutritional packages (Table 1) of various combinations of a varied number of mineral sources. The silkworm crop was raised, from the eggs of *Bombyx mori* L., a Pakistani strain, which were shifted to an incubator, for hatching at 30±1°C. The neonates, were, within two hours of their hatching, brushed into ninety card board boxes, of 24×18 inch size, in lots of 100 larvae, each, shifted to an air conditioned insect rearing Laboratory, run at 25±1°C, 75±2% R.H., using 16 h of a dim day light following Karishnaswami and Sundramurthy [6]. They were offered chopped fresh tender mulberry leaves, five times daily, up to the end of 2nd instar and later on, three times daily. These trials were laid out in a Completely Randomized Design, under factorial arrangements, having 10 treatments including a control with three repeats each, repeated for three years.

Data collection and analysis: Thus, the data on various developmental characteristics, comprised 6 estimates on the food consumption, coefficient of food utilization, larval body weight, larval body length, larval mortality percentage as well as on the larval duration, where as those on the biological aspects comprised 7 estimates, on the moth eclosion, crumpled wing individuals, mating duration, female adult life span, male adult life span, fecundity as well as those on the hatching percentage.

As such, the estimates on the developmental aspects, such as, those on food consumption and co-efficient of utilization, were calculated, at the end of each instar, by the following formulae, respectively.

F.C = Dry weight of the leaves offered - Dry weight of the left over leaves

and

$$C.U = \frac{\text{Dry wt. of the food consumed - Dry wt. of the faeces}}{\text{Dry wt. of the food consumed}} \times 100,$$

after Evan [7] and computed in cumulative terms, per larva.

The records on larval body weight and larval body length, were based on an average estimate of 10 larvae, taken at random, from each lot, at the end of each instar and computed in cumulative terms, per larva, in the same way, given above. The mortality percentage, was based on the daily number of dead larvae / treatment, whereas, the cumulative %age mortality was calculated, at the end of larval life and the total larval duration was computed, by adding up the larval duration of all the 5 larval instars.

The estimates on the biological aspects of this insect, were taken after the cocoon formation. For this purpose, 20 cocoons, from each treatment, were selected, as seed-cocoons and placed in a grainage-tray; in a single layer, at 25±1°C and 75±2% R.H., for moth emergence. Moth eclosion, started after 9 days of the cocoon formation and the eclosion percentage was calculated, as under;

$$\text{Moth eclosion percentage} = \frac{\text{No. of Pierced Cocoons}}{\text{Total No. of Cocoons}} \times 100$$

The percentage of crumpled-wing individuals was calculated, as given below.

$$C.W.I.\ \%age = \frac{C.W.I}{\text{Total No. of Moths emerged}} \times 100$$

Soon after eclosion, the pairing was encouraged, within the individuals of the treatment and their mating time, noted, in hours. Usually, the male moths died after mating and the female, after an egg-lay. Their adult life span was also recorded from the time of their eclosion to

Table 2: A multiple comparison of The mean-values of different nutritional sources or treatments (Ts), with those of various developmental and biological aspects of the silkworm, *Bombyx mori* L., larvae, at 25±2°C, in the Entomological Laboratories, University of Agriculture, Faisalabad
(Data on the comparative mean values)

Trts.	T. Mineral Matter Contents	Developmental aspects						Biological aspects						
		Food consumption (g)	Co-efficient of utilization (%)	Larval body weight (g)	Larval body length (cm)	Mortality (%)	Larval duration (d)	Moth eclosion (%age)	C.W. Individuals (%age)	Mating duration (h)	Adult life span (f) (days)	Adult life span (m) (days)	Fecundity (ns.)	Hatching (%age)
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
T_9	700.05	6.57**a	85.22**a	6.24**a	6.98**a	0.22d	31.63**h	77.41**a	0.23**e	7.00**a	8.25**a	7.81**a	450.20**a	89.07**a
T_8	631.05	6.52b	84.48b	6.19b	6.93b	0.67cd	31.78h	76.48a	0.47e	6.85a	8.07ab	7.57a	446.20a	88.37a
T_7	556.65	6.22c	80.23c	5.92c	6.71c	0.66cd	32.82g	72.59b	1.32de	6.24b	7.87bc	7.07b	406.50b	86.04b
T_6	527.40	6.12d	79.08d	5.81d	6.60d	0.66cd	33.00g	70.93bc	1.86cde	6.01bc	7.63cd	6.96b	397.80b	85.11b
T_5	428.10	5.88e	76.94e	5.61e	6.42e	0.37cd	33.63f	69.63c	1.63cde	5.77cd	7.35d	6.75bc	384.50c	82.89c
T_4	275.10	5.57f	74.10f	5.35f	6.14f	0.89cd	34.59e	67.04d	2.26cde	5.48d	7.05e	6.48c	360.00d	80.30d
T_3	220.05	5.11g	69.56g	4.99g	5.75g	0.89cd	35.44d	63.70e	2.86bcd	4.72e	6.27f	5.63d	322.60e	75.07e
T_2	119.70	4.88h	66.01h	4.61h	5.44h	1.11c	36.33c	61.30f	3.60bc	4.20f	5.90g	5.05e	287.20f	70.56f
T_1	64.20	4.60i	58.47i	4.30i	5.18i	1.86b	37.67b	58.33g	4.47b	3.57g	5.18h	4.49f	254.00g	66.11g
T_0	0.00	3.84j	44.50j	3.36j	4.55j	4.67**a	41.11a	53.89h	8.25a	2.75h	4.22i	3.87g	223.00h	61.30h

** = Highly significant at 1% level

the time of their death, in each case and the no. of eggs laid by each female, in each treatment, was also recorded by the visual egg counts and the eggs were placed in an incubator, at 30±1°C and 75±2% R.H., for hatching and the hatching percentage was calculated, by the following formula.

$$\text{Hatching \%age} = \frac{\text{No. of eggs hatched}}{\text{Total No. of eggs}} \times 100$$

The significance of the variance, between them, from one treatment to another, was calculated through the Duncan's Multiple Range Test, after Steel and Torrie [8]. Different statistical estimates on the correlations, of observed changes, with those of the mineral-doses, were calculated by running these datae, on an IBM Computer, using an M. Stat Programme and the Principal Component Analyses, for each character set, were sorted out, after Johnson and Wichern [9].

RESULTS AND DISCUSSION

The information collected on the effect of different nutritional sources on the developmental and biological aspects of the mulberry silkworm, larvae, is displayed through Table 2. The data, are presented in terms of a multiple comparison of the mean values of 13 different Parameters [Table 2 (2-14)] with those of the mineral matter contents [Table 2 (1)].

Thus, a perusal of the facts contained in Table 2, on the effects of different mineral sources with reference to the development and biology of the *Bombyx mori* L. larvae, shall reveal a highly significant change in the mean values, not only of the food consumption [Table 2 (2)], coefficient of food utilization [Table 2 (3)], body weight of larvae [Table 2 (4)], body length of larvae [Table 2 (5)], larval mortality [Table 2 (6)], larval duration [Table 2 (7)], moth eclosion [Table 2 (8)], crumpled wing individuals [Table 2 (9)], mating duration [Table 2 (10)], female life span [Table 2 (11)], male life span [Table 2 (12)], but also in those of the fecundity [Table 2 (13)] and hatching % [Table 2 (14)], from one treatment to another.

A perusal of the correlation matrix in Table 3, on the effect of different treatments-shall reveal that the changes in the larval mortality of the *Bombyx* larvae [Table 3 (5)] as well a those in the larval duration [Table 3 (6)], in addition to those in the crumpled wing individuals [Table 3 (8)], were found to be negatively correlated to the ones in the doses-that express the total mineral matter contents. On the contrary, however, the changes in food consumption [Table 3 (1)], coefficient of utilization [Table 3 (2)], larval body weight [Table 3 (3)], larval body length [Table 3 (4)], moth eclosion [Table 3 (7)], mating duration [Table 3 (9)], female adult life span [Table 3 (10)], male adult life span [Table 3 (11)], fecundity [Table 3 (12)] as well as those in the hatching %age [Table 3 (13)], were positively correlated with the changes in the doses, from one treatments to another.

The comparison of these studies with those of others, shall reveal that although the previous studies varied much, particularly in comparison with the present attempts, yet the work carried out by Patil *et al.* [2], Vishwanath *et al.* [3], Bhoopathy and Gunasegar [10] as well as Shankar *et al.* [4] and Jadhav *et al.* [5] can serve the purpose. Thus, the association of food consumption, co-efficient of utilization, larval body weight, larval body length, fecundity and hatching %age etc., with the total

Table 3: A correlation matrix between various test doses of the mineral matter (Ts) and the changes in various developmental as well as Biological aspects of the silkworm, *Bombyx mori* L., Larvae, at 25±2°C, in the Entomological Laboratories, University of Agriculture, Faisalabad
(Data From the Computer Listing)

S. No.	Characters		V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}	V_{13}
	Developmental aspects:														
	Doses	X													
1	Food Consumption	0.973**	1.00												
2	Co-Efficient of Utilization	0.926**	0.984**	1.00											
3	Body Weight	0.953**	0.995**	0.995**	1.00										
4	Body Length	0.971**	0.999**	0.985**	0.996**	1.00									
5	Mortality	-0.680**	-0.796**	-0.869**	-0.836**	-0.800**	1.00								
6	Larval Duration	-0.935**	-0.987**	-0.996**	-0.994**	-0.986**	0.856**	1.00							
	Biological aspects:														
7	Moth Eclosion	0.965**	0.974**	0.933**	0.966**	0.972**	-0.757**	0.664**	1.00						
8	C.W. Individuals	-0.805**	-0.878**	-0.903**	-0.893**	-0.874**	0.830**	-0.464**	-0.835**	1.00					
9	Mating Duration	0.965**	0.986**	0.972**	0.982**	0.988**	-0.789**	0.657**	0.977**	-0.861**	1.00				
10	A. Life Span (F)	0.952**	0.987**	0.983**	0.988**	0.990**	-0.815**	0.605**	0.963**	-0.865**	0.983**	1.00			
11	A Life Span (M)	0.960**	0.980**	0.964**	0.975**	0.983**	-0.773**	0.661**	0.973**	-0.847**	0.982**	0.986**	1.00		
12	Fecundity	0.980**	0.990**	0.967**	0.982**	0.991**	-0.756**	0.685**	0.974**	-0.860**	0.988**	0.981**	0.983**	1.00	
13	Hatching	0.967**	0.991**	0.976**	0.988**	0.994**	-0.780**	0.638**	0.968**	-0.864**	0.986**	0.986**	0.980**	0.990**	1.0

** = Significant at 1% level

Table 4: The regression coefficients / principal component (PC) analysis for the impact of different doses (X) or the mineral matter (Ts) on the individual (1 to 2) aspects, of various developmental and biological changes, in the silkworm, *Bombyx mori* L., larvae
(Data from the Computer listing)

S. No.	Parameters	Variable (A)	Regression equation (B)	F-Ratio (C)	R^2-Change (D)	PC-1 Proportion (E)
1	Developmental aspects:					
	Doses	X				
	Food Consumption	V_1	4.29+0.00352 X	501.55	94.7%	
	Coeff. of Utilization	V_2	55.2+0.0472 X	169.73	85.8%	
	Larval Body Weight	V_3	4.00+0.00354 X	278.44	90.9%	
	Larval Body Length	V_4	4.96+0.00317 X	468.54	94.4%	
	Mortality	V_5	2.51-0.00371 X	24.09	46.2%	
	Larval Duration	V_6	38.7-0.0111 X	195.10	87.4%	
	Overall		11.50+0.0300 X	171.30	86.0%	0.950
	The regression equation is:					
	PC1 = 11.5 + 0.0300 Dose					
	Where, PC1 = 0.413 V_1 + 0.418 V_2 + 0.417 V_3 + 0.414 V_4-0.368 V_5-0.417 V_6					
	Predictor	Coef.	Stdv.	t-ratio	P	
	Constant	11.4990	0.9719	11.83	0.000	
	Dose	0.029963	0.002289	13.89	0.000	
	S = 2.971		R-sq = 86.0%	R-sq (adj) = 85.4%		
2	Biological aspects:					
	Moth Eclosion %age	V_7	56.4+0.0306 X	384.87	93.2%	
	C-Winged Individuals %age	V_8	5.62-0.00829 X	51.53	64.8%	
	Mating duration	V_9	3.33+0.00550 X	376.17	93.1%	
	A. life span (f)	V_{10}	4.96+0.0516 X	268.33	90.6%	
	A. Life span (m)	V_{11}	4.33+0.00522 X	333.03	92.2%	
	Fecundity	V_{12}	244+0.310 X	678.73	96.0%	
	Hatching %age	V_{13}	65.1+0.0378 X	399.20	93.4%	
	Overall		-143-0.154 X	637.96	95.8%	0.953
	The regression equation is:					
	PCI = -143-0.154 Dose					
	Where, PC1 = -0.379 V_{19} + 0.346 V_{20}-0.384 V_{21}-0.383 V_{22}-0.383 V_{23}-0.384 V_{24}-0.384 V_{25}					
	Predictor	Coef.	Stdv.	t-ratio	P	
	Constant	-143.034	2.591	-55.20	0.000	
	Dose	-0.154179	0.006104	-25.26	0.000	
	S = 7.922		R-sq = 95.8%	R-sq (adj) = 95.6%		

** = Significant at 1% level and a blank () = non-significant

Table 5: The statistical impact of the individual (1 to 2) changes, in various developmental and biological aspects of the silkworm, *Bombyx mori* L., on their silk produce, after being supplemented with different nutritional sources (Ts)
(Data from the Computer listing)

					R^2-Change for	
		Variables	B-Values	T-values	Characters	Sets
S. No	Parameters	(A)	(B)	(C)	(D)	(E)
1	Developmental aspects:					
	Yield	Y				
	Larval Body Weight	V_3	0.11263	6.175	0.929422321	
	C. Utilization	V_2	0.002666705	2.286	0.059437678	
	Intercept		0.58950	18.533		
	Overall			1197.97912		0.98886
2	Biological aspects:					
	Hatching %age	V_{13}	0.007616643	4.815	0.120260172	
	A. Life Span (F)	V_{10}	0.03777	3.309	0.868139828	
	Intercept		0.61137	12.316		
	Overall			1150.36188		0.98840

mineral matter contents (Doses), was found to be exactly similar to that reported by the previous workers, where as, the association of moth eclosion, crumpled wing individuals larval mortality, female adult life span and male adult life span as well as of the mating duration was being reported for the first time, in view of the reviewed literature.

The data on the magnitude of changes are laid out in Table 4, which pertains to the information on the regression co-efficient / Principal Component (P.C.) Analysis. It shall reveal an overall PC-1 proportion of 0.950 for the developmental aspects [Table 4 (E)] and 0.953 for the biological aspects of the silkworm [Table 4 (E)]. On comparing the overall situation, between the 2 subsets, under discussion, the overall R^2 (adj) -values of 85.4% [Table 4 (D)] and 95.6% [Table 4 (D)] for the developmental aspects as well as for the biological characteristics of the *Bombyx mori* L. respectively, shall reveal that changes in the mineral matter contents, has affected the various biological aspects in a little better way than the developmental aspects.

As to the statistical impact of various, changes in different aspects of silkworm life, the data in Table 5 shall reveal an R^2-value for different character sets to be 0.9888 [Table 5 (E)] and 0.9884 [Table 5 (E)] with references to the developmental aspects and bionomical characters, respectively. This suggests, in brief, that the changes in the developmental as well as in the biological aspects of the silkworm, had affected the silkworm life to an almost a similar degree.

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