Nigerian Journal of Ecology (2014) 13:19-26.

ISSN: 1116-753X

Developmental and Cocoon Parameters of the Silkworm *Bombyx mori* L. (Lepidoptera: Bombicidae) fed on Mulberry Leaves Preserved for Different Durations under Tropical Conditions

Alebiosu, I.B[a]., Olatunde G.O[b]., Adedire, M.O[c]. and Pitan O.O.R[d]*
[a]Forestry Research Institute of Nigeria, Ibadan, Nigeria
[b]Osun State University, Osogbo, Nigeria
[c]Department of Wildlife and Forestry, University of Agriculture, Abeokuta, Nigeria
[d]Department of Crop Protection, University of Agriculture, Abeokuta, Nigeria

*Corresponding author: femipitan@yahoo.com

(Accepted 19 April 2014)

ABSTRACT.
The general performance and cocoon yield of two silkworm hybrids fed on mulberry leaves preserved for 0, 24, 48, 72 and 96 hours were investigated in a 2 x 5 factorial arrangement fitted into a completely randomized design with 4 replicates. The aim of the experiment was to identify the best of the four leaf preservation durations for cocoon production optimization. Each replicate consisted of one hundred silkworm larvae of each hybrid which were fed throughout the larval stages on leaves preserved at different durations. Leaf samples obtained from each preservation duration were analyzed for nutrient contents. Results showed that the shell ratio, number of cocoons and cocoon weight were significantly influenced by preservation duration and silkworm hybrids. Single cocoon weight and shell weight were significantly higher at 0 and 24 hours preservation duration compared to others. Fecundity, larval and pupal emergence were not influenced by the two factors. However, higher larval and pupal weights were observed when silkworms were fed on leaves preserved for 0 and 24 hours. Compared to other preservation durations, fresh leaves and those preserved for 24 hours gave the best cocoon quality.

Keywords: silkworm, *Bombyx mori*, feeding, leaf preservation, cocoon, larva

INTRODUCTION

The silkworm (*Bombyx mori*) had been domesticated for over four thousand years exclusively on mulberry leaves (He, 2010). Since the sole food for the growth of silkworm is mulberry leaves (Arunkumar *et al.*, 2006; He, 2010), adequate supply and good quality mulberry leaves are therefore essential for the production of quality cocoons. Silkworm rearing is labour intensive hence preserving leaves for subsequent use may be inevitable. Leaf preservation may be a very important management practice during silkworm rearing especially during inclement weather conditions when it may be difficult to go out to get the leaves or where mulberry fields may be far from the rearing house. In preserving mulberry leaves, it must be ensured that the leaves are not preserved longer than necessary in order to avoid wilting which reduces or causes a complete loss of the quality required for silkworm development. This is because generally,

dietary water is very important in silkworm rearing, and leaves loose moisture and nutrients as the length of preservation increases (Narasimhamurty *et al.,* 1987; Prem and Bongale, 1989).

Moisture from mulberry leaves plays a major role in silkworm development since the sole source of water for silkworm is from mulberry leaves (Esfandarani *et al.,* 2002). Previous studies by Friend (1958) and Waldbaner (1968) showed that feeding phytophagous insects with wilted leaves had a negative effect on the physiology of insects. Decrease in water content of mulberry leaves fed to silkworm affects different energetic parameters and reduces the assimilation of food converted to silkworm body (Paul *et al*., 1992). Cocoon shell and pupal weights including fecundity have also been recorded to increase with increase in leaf moisture content (Arunkumar, 2006).

However, apart from loss of moisture, other nutrients like protein and carbohydrates reduced after two hours of preservation (Narasimhamurty, 1987). This decrease in protein contents of mulberry leaves was attributed to proteolysis due to oxidation during respiration. Since leaf preservation may not be unavoidable, it is therefore imperative to determine how long mulberry leaves should be preserved in order to obtain maximum cocoon yield when fed subsequently to silkworms. In this study therefore, we investigated the general performance and cocoon yields of two hybrids of silkworm fed on mulberry leaves that were preserved for different durations under tropical conditions.

MATERIALS AND METHODS

Experimental set-up

The study was carried out in the Sericulture Laboratory, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The eggs of the five silkworm species used were obtained from Ekiti State Sericulture Centre Ado-Ekiti, Nigeria, while the mulberry plant variety S30 accessioned in FRIN was used for the feeding of the silkworm species. The experiment consisted of two factors: (a) 2 hybrids of the silkworm – W_1D_2 and C_1J_2 (b) 5 leaf preservation periods - 0 hr, 24 hrs, 48 hrs, 72 hrs and 96 hrs, and was conducted using a 2 x 4 factorial arrangement fitted into a completely randomized design with four replicates. The mulberry leaves were harvested between 8 and 10 am and preserved in transparent polythene bags for the different preservation periods at ordinary room temperature and humidity (28±2^{o}C; 70%RH) for 19-21 days. Leaf samples from each treatment were randomly taken from the preserved mulberry leaves. The samples were weighed, oven-dried to constant weight and allowed to cool. They were thereafter ground to fine powder and analyzed for nutrient contents as described by the Association of Official Analytical Chemist (AOAC, 1998).

Silkworm is known to react negatively to environmental conditions, especially temperature and relative humidity. These factors were closely monitored during the cause of these experiments and maintained at 23-26^{o}C and 73-86% RH using the standard suggested by Ullal and Narasimhanna (1987) and Suresh *et al*. (2007).

Preparation of eggs for hatching

Diapausing eggs of the five silkworm hybrids were preserved in refrigerator at 2.5-5^{o}C. The eggs were removed from the refrigerator as need arose. Prior to the removal of the eggs for rearing, the temperature of the refrigerator was raised from 5 to 15^{o}C for 3 hours to avoid shock. Thereafter, the egg cards were rinsed in 2% formalin solution for 15 minutes and later washed in running water. The egg cards were then kept between two large sheets of

filter paper to dry and subsequently immersed in glass trough of Hydrochloric acid (HCl) (1.10 specific gravity) at 29^{o}C for 50 minutes (cold acid treatment) to break the diapause (Jolly, 1988). Thereafter, the eggs were removed from the acid and rinsed under running water for 5 minutes until the acid was completely washed off. For incubation, the egg cards were then turned upside down on large paper spread in a rearing tray on which old newspaper sheets were spread.

Twenty four hours later, previously disinfected foam pads were soaked in water and arranged at the four sides of the rearing tray before covering with paraffin or nylon paper. This was to maintain high humidity and temperature for 10-11 days, while the colour change of the eggs was observed. When eggs were freshly laid, they were yellow in colour but turned brown 24 hours later ushering in hibernation and it was at this stage they were acid-treated. When the egg colour turned blue or at the egg pigmentation day, the egg cards were kept in total darkness for 2 - 3 days, a process called 'black boxing'(Jolly, 1986). On the 11th day of incubation, the eggs were exposed to bright light early in the morning. This was done to achieve uniform hatching because darkness arrests hatching of the developed eggs and facilitates lagging embryo to reach hatching phase. The egg cards were spread uniformly in one layer on paraffin paper.

Brushing of newly hatched eggs

After two hours of exposing the eggs to light in the morning, chopped leaves (0.5 cm x 0.5 cm) were sprinkled in a single layer over the hatched larvae for about 10-20 minutes to allow the larvae to crawl on the cut leaves. The egg sheets were turned upside down and held about 5 cm above the rearing tray with paraffin paper and taped gently to complete the process of transferring the larvae (brushing).

Preparing rearing house and equipment

Five days to the commencement of brushing, the rearing room, rearing trays, and all other equipment were washed, dried and disinfected with 3% formalin solution. After brushing, one hundred silkworm larvae were randomly selected from each of the five hybrids and fed four times at 8.00 hrs, 11.00 hrs, 14.00 hrs, and 17.00 hrs on preserved leaves at different durations until they passed the five larval stages. Treatment trays were randomly distributed on the rearing racks, and thereafter, larval mortality was determined.

Cleaning and caring for the worms

To maintain hygiene in the rearing bed, dirt such as left-over or rejected leaves, fecal matters and carcasses of the third, fourth and fifth instar larvae were removed from the rearing trays on a daily basis, and covered with clean nets. Mulberry leaves were broadcast on the net after the cleaning procedures, making the larvae to climb onto the net from the trays and hang onto them while trying to reach and feed on the fresh leaves. The larvae on the net were then transferred into other clean trays or cleaned old trays, and thereafter, clean sheets of paper were spread on them. This procedure was not carried out on the first and second instars which were fragile so as not to lose them.

At the commencement of each moulting, the larvae were dusted with slaked lime ($CaCO_3$) to dry up the bed and leaves so that all the larvae could stop feeding. Dusting also reduced the chances of the growth of mould and other disease-causing micro-organisms

Mounting of mature worms and cocoon processing

A day before the commencement of cocoon formation, fully grown silkworm larvae were later mounted on montages for cocoon spinning. All cocoons were harvested at six

days old, out of which 20 were randomly selected from each replicate for the determination of pupal weight, shell weight and shell ratio. The pupae were subsequently kept until the adults emerged. Percentage adult emergence was calculated using the formula:

$$\frac{\text{Number of emerged adults}}{\text{Total number of pupae.}} \times 100$$

Freshly emerged male and female adults were placed on rearing trays for mating for three hours according to the different treatments in four replications. The females were later separated and put in cellule on thick brown cardboard paper where they laid their eggs. After 24 hours, newly laid eggs were acid–treated and incubated, and the newly hatched larvae were brushed (Ullal and Narasimhanna, 1987)

The number of hatched eggs was determined from the number of egg shells recovered. After hatching, the egg shells became white, un-hatched eggs became black, while unfertilized eggs retained their yellow colour. The number of hatched eggs was therefore calculated as percentage of the total eggs laid. Other data taken were: larval mortality, larval developmental period, larval weight, pupal weight, percentage larval emergence, and fecundity, In order to determine longevity of adult silkworm, five pairs (males and females) were kept separately in Kilner jars till death, and number of days from adult emergence to death represented longevity. The following formulae were used to calculate the data taken:

Larval developmental period = No of days from hatching to cocooning;

Larval weight = average weight of 10 larvae;

Pupal weight = average weight of 10 pupae;

Percentage larval emergence

$$= \frac{\text{No of emerged larvae}}{\text{Total number of eggs,}} \times 100$$

Fecundity = Total no of eggs laid by one adult female;

All data collected were subjected to analysis of variance (ANOVA) test at $P < 0.05$. Least Significant Difference (LSD) was used to compare the means. The concentrations of the chemical constituents of the different preserved mulberry leaves were correlated with the cocoon yield parameters and developmental characteristics of the silkworms.

RESULTS

Shell ratio was significantly influenced by leaf preservation duration and/or silkworm hybrids, while number of cocoons and single cocoon weight were jointly influenced by two factors. Irrespective of silkworm hybrid, single cocoon weight was significantly higher at 0 - and 24-hour preservation durations compared to others (Table 1). Shell weight was significantly higher when silkworm hybrids were fed on unpreserved leaves and leaves preserved for 24 hours. Although, number of cocoons produced was inconsistent, the value was significantly higher with hybrid C_1J_2 at 0, 24 and 96 hours, which were not significantly different from one another. Shell ratio was higher when silkworm hybrid C_1J_2 was fed on leaves preserved for 72 and 24 hours compared to others or with either species at other preservation durations (Table 1). Fecundity, larval and pupal emergence were not influenced by the two factors (Table 2). However, higher larval and pupal weights were observed when silkworms were fed with leaves preserved for 0 and 24 hours relative to others.

Whereas crude fat content was significantly higher ($P < 0.05$) in unpreserved leaves and decreased significantly ($P < 0.05$) as the

duration of preservation increased, crude protein content was higher in leaves preserved for 96 hours and decreased significantly ($P < 0.05$) with the length of preservation (Table 3). Crude fibre and total ash contents were higher in unpreserved leaves and those preserved for 24 hours than at other durations (Table 3). Soluble carbohydrate content was significantly higher ($P < 0.05$) at 96 hours followed by 72 hours, while the least soluble carbohydrate content was found at 24 hours of preservation and unpreserved leaves, which were not significantly different from each other. Moisture content was significantly higher in leaves preserved for 72 hours relative to other preservation periods (Table 3).

There was a negative correlation between crude protein content and number of cocoons, single cocoon weight, pupal weight and larval weight, and positive with shell weight. However, a significant positive correlation existed between crude fat content and number of cocoon, single cocoon weight, shell weight, laval and pupal weights. Similarly, total ash content was positively correlated ($P < 0.05$) with number of cocoon, single cocoon weight, shell weight, laval and pupal weights, whereas nitrogen-free extractives were negatively correlated with number of cocoons, single cocoon weight, shell weight, laval and pupal weights (Table 4). Shell ratio and pupal weight have a positive relationship with dry matter, while moisture content and shell ratio were also positively correlated (Table 4).

Table 1: Cocoon yields of two silkworm hybrids fed with mulberry leaves preserved at different durations

Variables	Silkworm Hybrid	Preservation durations (hours)					Mean
		0	24	48	72	96	
Number of Cocoons	C_1J_2	89.8	87.5	76.0	75.8	93.5	80.5
	W_1D_2	80.0	81.0	78.3	82.3	72.3	78.8
	Mean	84.9	84.3	77.1	79.0	72.9	
		$LSD_{(0.05)}$ S = 3.5; P = 5.5*; SxP = 7.8*					
Single cocoon weight (g)	C_1J_2	1.4	1.3	1.2	1.1	1.0	1.2
	W_1D_2	1.3	1.3	1.2	1.0	1.2	1.2
	Mean	1.4	1.3	1.2	1.1	1.1	
		$LSD_{(0.05)}$ S = 0.05; P = 0.07*; SxP = 0.10*					
Shell weight (g)	C_1J_2	0.28	0.30	0.24	0.27	0.22	0.26
	W_1D_2	0.26	0.26	0.25	0.23	0.20	0.24
	Mean	0.27	0.28	0.24	0.25	0.21	
		$LSD_{(0.05)}$ S = 0.01*; P = 0.02*; SxP = 0.03					
Shell ratio	C_1J_2	19.6	24.7	20.0	25.0	22.6	22.3
	W_1D_2	19.8	20.3	20.8	22.6	17.0	20.1
	Mean	19.7	22.3	20.4	23.8	19.8	
		$LSD_{(0.05)}$ S =1.4*; P = 2.2*; SxP = 3.2*					

Values are means of four replicates. LSD's are for the following comparisons. S, Silkworm hybrids; P, Preservation duration; SxP= Interaction between Silkworm hybrids and Preservation duration; * significant at $P < 0.05$.

Table 2: Developmental characteristics of two silkworm hybrids fed with mulberry leaves preserved at different durations

Variables	Silkworm hybrid	Preservation duration (hours) 0	24	48	72	96	Mean
Larval weight (g)	C_1J_2	2.9	2.88	2.65	2.81	2.55	2.76
	W_1D_2	2.93	3.13	2.86	2.72	2.87	2.90
	Mean	2.91	3.00	2.75	2.76	2.71	
		$LSD_{(0.05)}$ S = 0.11*; P = 0.17*; SxP = 0.24*					
Pupal weight (g)	C_1J_2	1.05	0.95	0.90	0.82	0.75	0.89
	W_1D_2	0.97	0.94	0.89	0.82	0.96	0.92
	Mean	1.00	0.95	0.89	0.82	0.86	
		$LSD_{(0.05)}$ S = 0.04; P = 0.06*; SxP = 0.09*					
Fecundity	C_1J_2	427	508	410	424	415	437
	W_1D_2	414	415	381	405	387	400
	Mean	420	461	396	414	401	
		$LSD_{(0.05)}$ S =40.6; P =64.1; SxP = 90.7					
Larval emergence (%)	C_1J_2	96.7	98.5	97.1	99.1	93.4	97.0
	W_1D_2	96.1	97.1	96.7	95.3	95.2	96.1
	Mean	96.4	97.8	96.9	97.2	94.3	
		$LSD_{(0.05)}$ S =2.3 ; P =3.6 ; SxP =5.2					
Adult emergence (%)	C_1J_2	100	100	100	100	100	100
	W_1D_2	100	100	100	100	100	100
	Mean	100	100	100	100	100	

Values are means of four replicates. LSD's are for the following comparisons. S, Silkworm hybrids; P, Preservation duration; SxP = Interaction between Silkworm hybrids and Preservation duration; *, Significant at $P < 0.05$

Table 3: Proximate compositions of mulberry leaves preserved for different durations

Proximate composition (%)	Preservation duration (hours) 0	24	48	72	96	$LSD_{(0.05)}$
Crude fat	5.63	5.28	5.31	4.73	4.18	0.15*
Crude protein	16.72	17.29	17.07	17.24	17.13	0.22*
Crude fibre	14.35	14.41	14.09	13.70	13.46	0.25*
Total ash	13.14	13.25	12.95	11.86	11.38	0.26*
Soluble carbohydrate	50.38	50.16	50.39	52.16	53.10	0.49*
Dry matter	90.10	89.81	89.93	88.32	90.15	0.28*
Moisture content	9.90	10.19	10.07	11.68	10.18	0.28*

Values are means of three replicates; *, significant at $P < 0.05$.

Table 4: The coefficients of linear correlation between the proximate constituents of mulberry leaves preserved for different durations and cocoon yields and silkworm developmental characteristics.

Parameter	Number of cocoons	Single cocoon weight	Shell weight	Shell ratio	Larval weight	Pupal weight	fecundity	Larval emergence
Crude protein (%)	-0.793*	-0.722*	-0.656*	0.021	-0.544*	-0.529*	-0.132	-0.231
Crude fat (%)	0.584*	0.627*	0.634*	0.035	0.458*	0.443*	0.136	0.353
Crude fibre (%)	0.079	0.122	0.054	0.059	0.213	0.007	-0.199	0.226
Total ash (%)	0.655*	0.691*	0.597*	0.005	0.487*	0.438*	0.185	0.328
NFE (%)	-0.625*	-0.663*	-0.605*	-0.063	-0.463*	-0.414*	-0.221	-0.369
Dry matter (%)	0.169	0.191	-0.098	-0.565*	0.154	0.471*	-0.088	-0.104
Moisture (%)	-0.209	-0.211	0.017	0.538*	-0.168	-0.491*	0.047	0.080

* = Correlation is significant at 0.05 level, NFE= Nitrogen Free Extract

DISCUSSION

Silkworm rearing is labour intensive because of the number of times the larvae need to be fed daily. This situation therefore, makes leaf preservation inevitable under a sustainable silkworm rearing for cocoon production. This is particularly important in situations when it could rain for days and it would become difficult to go out for leaf-harvesting in order to feed the worms. This situation can be a mitigating factor in silkworm rearing. Again, when sericulture production is combined with other businesses, leaf preservation becomes necessary. From this study, when silkworm were fed with freshly-plucked leaves or 0-24 hours old leaves, cocoon number, single cocoon weight were significantly higher compared to other preservation periods. The yield parameters in fact, reduced with increase in preservation periods. This indicates that the quality of the mulberry leaves reduced with increasing duration of preservation. In the same vein, Prem and Bungale (1989) reported a significantly lower rate of loss in moisture content and a drop in leaf quality when polythene cover was used for mulberry leaf preservation.

In this study, it was observed that protein and carbohydrate contents increased and that of moisture content and crude fat reduced with leaf preservation periods, unlike in a similar study, (Narasimhamurty *et al.*, 1987) where protein and carbohydrate contents of mulberry leaves decreased after 12-hours of preservation**.** This finding therefore suggests that the decrease in cocoon yield observed in this study might not be due to loss in protein and carbohydrate content but rather due to other factors probably not investigated.

Leaf preservation in large-scale silkworm rearing is certain so as to save cost and labour. It is particularly important under our climatic condition especially during unfavourable weather conditions. Since results obtained from 48- and 72-hour leaf preservation regarding cocoon, shell, larval and pupal weights and fecundity still fall within the acceptable international yield standard when compared with what obtains in China and Japan (Krishnaswami *et al.*,1972), leaf preservation can still be carried out up till 48- and 72-hour when it cannot be helped.

REFERENCES

Association of Official Analytical Chemists (AOAC). (1998). Official Methods of Analysis. 11th Edition. Washington D.C.

Arunkumar, K. P., Muralidhar, M. and Nagaraju, J., (2006). Molecular phylogeny of silkmoths reveals the origin of domesticated silk moth *Bombyx mori* from Chinese *Bombyx mandarina* and paternal inheritance of *Antheraea proylei* mitochondria DNA. Molecular Phylogenetics and *Evolution* **40 (2):** 419-427.

Esfandarani, M. T., Bahreini, R. Tajabadi, N. (2002). Effect of mulberry leaves moisture on some traits of the silkworm *Bombyx mori* L. *Sericologia* **42 (2):** 285-289.

Friend, W.G. (1958). Nutrition requirements of phytophagous insects. *Annual Review of Entomology*, **3:**57-73

He, G. H., Wu, P. Z. and Huang Y. G. (1989). Economic efficiency of scarce rearing of *Bombyx mori* L. *Seridok* **5(3,4):** 129-130.

Jolly, M. S. (1986a). Economics of sericulture under rainfed conditions. Sericulture. Central Sericultural Research and Training Institute, Mysore, India. Project 1. Page 19.

Kherdeker, R. C., Munde, A. T., Neharkar, P. S. and Sonkamble, M. M. (2000). Effects of Fertilizers on Foliage Yield of Mulberry, on *Morus alba* L. and Economic Traits of Silkworm *Bombyx mori* L. *Journal of Soils and Crops.* **10 (2):** 221-225

Krishnaswami, N. R., Rao M. and Suryanarayan, T. S. (1972). Sericulture manual: Silkworm Reeling FAO Agric Services Bull 15/3

Narasimhamurty, C. V., Donatus E., and Pillai S. V. (1987). Chemical composition of mulberry during preservation and leaf storage. *Sericologia* **27 (4):** 623-627.

Paul, D. C, Rao, G. S., Deb, D. C. (1992). Impact of dietary moisture on nutrition indices and growth of Bombyx mori L. and concomitant larval duration. *Journal of Insect Pathology*, **38 (3):** 229-235

Prem G and Bongale, U. D. (1989). Leaf preservation in Mysore local and Kanva-2 varieties of mulberry. *Indian Silk 27 (7):* 14-15.

Waldbauer, G. P. (1968). The consumption and utilization of food by insects. *Advanced Insect Physiology*., **5:** 229-288