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Performance of *Bombyx mori* and *Samia cynthia ricini* silkworms under controlled environmental conditions in Uasin Gishu County

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Abstract
The domesticated silkworm *Bombyx mori* and Eri worm (*Samia cynthia ricini*) are bivoltine and multivoltine, feeding exclusively on mulberry and castor leaves, respectively. The domestication of silkworms has made them highly sensitive to fluctuations in temperature and relative humidity. The research determined the performance of silkworm reared inside pre-constructed structures with equal dimensions (4 m x 4 m x 3 m height), with iron roof; timber walled (L1) and mud walled (L2). Greenhouses with four flaps open (L3), three sides open (L4), two sides open (L5), one side open (L6) and completely enclosed (L7), and concrete walled (L0) were used. The survival percentage, larval duration and cocoon quality and quantity were tested and compared. The temperature and relative humidity were recorded. The mean temperature of structures during rearing ranged between 22.73 °C±1.86 (L0) and 31.63 °C±0.81 (L7) during the wet season with a similar trend in the dry season, the mean relative humidity of 33.26%±7.29 (L1) and 43.18%±9.53 (L0) during the wet season and 33.11%±7.27 (L1) and 42.16%±7.38 (L7). Larval duration was longest in L2 for both *B. mori* and Eri during the wet season. Larval survival was highest in L2 (76.73±4.20%) and L0 (78.70%) during the wet and dry season respectively. A similar trend obtained in L2 (77.00%) and L0 (80.10%) for Eri in wet and dry season respectively. The cocoon weight was highest in structure L2 (0.86±0.03) during the dry season, L5 (2.78±4.30) during the wet season. Eri cocoon weight was highest in L0 (2.35±0.49) during the wet season and highest in L3 (2.44±0.34) in the dry season. The longest significantly different Filament length for *B. mori* was in L2 (1377.80±150.17 m). During the dry season L3 (1382.80±117.23m) was the longest. For the Eri the longest filament length was from L2 (437.6±32.26). The seasons did not influence average survival, filament length and weights from the tested structures.

Keywords: Eri, *B. mori*, survival, quality cocoons

Introduction
The silkworms *Bombyx mori* and Eri worm (*Samia cynthia ricini*) produce silk from the silk glands located in their head of mature larvae by forming a runny fluid before spinning a cocoon, the raw material for the production of silk (Zhou *et al.* 2022). In Kenya Eri worms (*Samia cynthia ricini*) a non-mulberry and mulberry *Bombyx mori* are fully domesticated silkworm species (Sharma and Kalita, 2017) [18]. The adult female moths lay eggs which hatch after being fertilized by the male moth of which the eggs hatch to larval stage. The worms can be reared throughout the year depending on the presence of host plants and the prevailing conducive environmental conditions.
According to Gong *et al.* (2020) [4], adverse environmental conditions occur regularly and the way in which it can affect the development of the organism varies. Further, the regulation of these factors can improve silkworm crop (Rahmathulla, 2012) [29]. The optimum temperature for normal growth of silkworms is between 24 °C and 28 °C and relative humidity of 75% are desirable for maximum productivity (Nguku *et al.* 2009) [12]. Controlled conditions are necessary to optimize temperature and humidity in regions like Uasin Gishu where temperatures range between 8.4ºC and 27ºC and relative humidity of below 45%, which are not suitable for rearing silkworms (gong *et al.* 2015; Kiplagat *et al.* 2022) [26, 7]. The rise in temperature increases various physiological functions in silkworms due to increased enzymatic activities and with a fall in temperature, the physiological activities reduce.

Increased temperature during silkworm rearing particularly in late instars accelerates larval growth and shortens the larval period. But at low temperature, the growth is slow thus prolonging larval period. During the first, second and third instars silkworms require high temperature and the worms feed actively, grow rapidly, leading to high growth rate. Such vigorous worms can withstand adverse conditions in later instars and therefore yield good quantity and high-quality cocoons such as cocoon weight and cocoon shell ratio (Sarkhel *et al.* 2017) [18]. Similarly, the relative humidity equally affects the vigour and survival of silkworms. The research sought to address this research gap by trying to manipulate temperature and humidity through rearing silkworms in controlled conditions for sustainable cocoon production in Uasin Gishu County.

Materials and Methods

The investigation to establish the suitability of sericulture (silkworm rearing) in Uasin Gishu was done by set up of rearing structures in university of Eldoret Zoology research site. The structures of equal dimensions coded L0-L7 (4mx4m and 3m high) each were constructed. L1 was a timber walled structure while L2 mudwalled, both structures were roofed with iron sheets of gauge 28. Four Greenhouses with four removable flaps were also set-up, greenhouse with all four flaps open (L3), 3 Flaps open (L4), 2 flaps open (L5), 1 flap open (L6) and all flaps closed (L7).

Greenhouses were made of polythene gauge 0.08mm/8mil/200 micron on all sides, the flaps were all covered with plastic nets (aperture 20Mtr weave type hexagonal, Mumbai, India) to prevent entry of predators. In each of the rearing structures, the rearing table made of timber was erected to hold the trays. The rearing tables were dipped in ant-wells to prevent insects from climbing the tables and attacking the silkworms. The timber house (L1) was a control representing conditions in Uasin Gishu.

Plate 1(a): Timber walled, mud walled and green house with all flaps open (c) Greenhouse structures L4, L5, L6, L7.

Survival and duration of silkworm larva under different conditions

To determine the suitable structure for the rearing of silkworm in Uasin Gishu, the pre-constructed structures Plate 1 were used. The larvae used to test duration and survival were obtained after uniform hatching of eggs in thermo incubator. In each condition 200 hatched larvae in the 2nd instar with three replicates were reared in wooden trays (1m x 0.5m). The worms were fed thrice per day at 9.00 am and at 1.00pm and 5.00pm with equal amount of succulent freshly plucked leaves of mulberry for *Bombyx mori* and castor leaves for Eri worms (Sharma *et al.* 2018) [19]. The temperature and relative humidity were recorded in the morning, afternoon and evening, just before feeding the worms while at night the hygrometer/thermometer recorded the minimum and maximum conditions. The duration to every instar and survival to the next instar of the population under each condition was recorded daily until pupation and cocooning.

During rearing to obtain clean beds a net was spread over the clean fresh leaves on a single layer spread, worms scrawled to fresh leaves and the ones left were handpicked, then transferred using the net to another clean tray and fed. The waste was removed from the trays into a compost bucket and disposed. The larvae were allowed to complete its instar stage in the structures. Similarly, the time to the next moult was recorded as the duration of the larval instar in days. The number of surviving larvae in each instar was recorded and the percentage survival calculated using the formula;

$$\text{Percentage survival} = \frac{\text{Total number of larvae moulted to the next instar}}{\text{Total number of larvae at the start of instar}} \times 100$$

Seasonal variations

Larvae obtained after uniform hatching of eggs in incubator were reared in both wet season (July-September) 2020 and dry season, (December, 2020 – February, 2021) using the same number of eggs and the same rearing procedures for comparisons.

Mounting

Mature silkworm cocoons at 5th instar showing maturity characteristics such as stopped feeding and crawl restlessly in search of a corner to attach itself, their abdomen appearing full of silk and shrunk in size were transferred from rearing beds into the area below the plastic mountages to start spinning. Observations were done continuously to ensure timely transfer. As the worm began to spin they were not disturbed since disturbance causes it to lay a spinning foundation afresh which means loss of some silk.

The cocoons were harvested on the 7th day from inception of spinning by carefully handpicking. At the end of spinning the larvae transformation into pupae was confirmed by cutting a randomly selected cocoon to observe if the pupa is brown in colour and hard. The harvested cocoons from every treatment were counted separately and recorded.

Assessment cocoon quality and quantity

From each structure ten cocoons from each tray (replicates) were sampled for analysis of cocoon quantity and quality based on the following parameters cocoon weight, cocoon quality, shell weight and shell ratio.

All measurements were done using high precision weighing balance of pinnacle brand model with accuracy of 0.01 mg/0.1 mg (Engineering Corporation Company).

Cocoon quality was determined by picking 10 cocoons from each replication and sorted out by removing the defective cocoons classified as (a) Double cocoons (b) Pierced cocoons (c) inside stained cocoons (d) Flimsy cocoons (e) Pointed or constricted cocoons (f) Outside stained cocoons (g) Flimsy cocoons, according to the reeling and testing manual (Lee, 1999) [10].

$$\text{Percentage of defective cocoons} = \frac{\text{Total number of defective cocoons}}{\text{Total number of cocoons per treatment}} \times 100$$

For the Cocoon weight, ten cocoons were picked randomly from pupated cocoons as described by Zulfiqar *et al.* (2022) [28], in each of the three replications and each cocoon was weighed in grams using high precision weighing balance described above. The weighed cocoons were then cut longitudinally on the side so that the pupa could be removed and weighed, with a lot of care not to harm the pupa. Further, the weight of the shell was determined by subtracting the individual weight of the pupa from the cocoon weight it was obtained from, to provide the ratio of the shell which carries the silk and the size of silk.

Shell weight = Whole cocoon weight-pupa weight.

$$\text{Shell ratio} = \frac{\text{weight of cocoon shell in grams}}{\text{Weight of the whole cocoon}} \times 100$$

Determination of filament length

Degumming was done to break the peptide bonds of sericin (Chattopadhya, 2017). The standard procedure of degumming of cocoons was followed. The *B. mori* cocoons were degummed using a two-pan cooking process (Chattopadhyay *et al.* 2018) [2] which involved putting the cocoons in a perforated cage, then immersed in the first cooking pan with water having temperature between 60-70ºc for about one minute, the cage was then transferred to the second cooking pan at 90ºc for about 2 minutes after which it was allowed to stand for a minute, cold water was sprinkled on the second pan to reduce temperature from 95ºC to 75ºC for 4 minutes. The cage was then opened in the water and cocoons brushed using a straw brush to produce a single filament.

The Eri cocoons were degummed by dipping the cocoons in degumming solution containing 10% sodium carbonate and 10% neutral soap and boiled for one hour (Chattopadhyay *et al.* 2018) [2]. Sodium carbonate was further used to produce a fibre breaking elongation to allow uniform degumming without significant deterioration of single fibre tenacity. The degummed cocoons were then put in a spinning wheel to separate filaments thereafter the filament length was measured using a tape measure.

Data Analysis

All data generated from the experiment was entered into the excel spreadsheet for the purpose of management. Analysis was done using Stratigraphic Centurion XVI and all values below 5% ($P \leq 0.05$) were designated as significant. The data that showed skewedness was first log transformed then Analysis of variance (ANOVA) was done to compare differences in means of temperature, humidity and larval duration, larval survival and cocoon parameters both in quantity and quality per structure, per species per season. A chi-square test was done on cocoon quality, a post hoc test was used to separate the means using Fisher's least significant different.

Results

Temperature and humidity of the experimental structures during the rearing of silkworm in wet and dry seasons

The highest mean temperature in °C was recorded in L7 (31.62±0.81), while the lowest mean temperatures were recorded in L0 (22.73±1.86) (Table 1). The temperatures in the eight structures showed significant variations ($F_{0.05\ (7,\ 368)} = 334.77$, $p<0.0001$). Significant difference was noted between L0 and L1, L3, L4, L5, L6 and L7. In the dry season, the highest mean temperature was recorded in structure L7 (31.52±0.74) °C followed by structure L6 (29.34±0.85) °C, while the lowest mean temperatures were recorded in structure L0 (22.86±2.20) °C with a significant difference ($F_{0.05\ (7,\ 368)} = 294.26$, $p<0.0001$). Significant difference was noted between L0 and L1, L3, L4, L5, L6 and L7. L3 differed significantly from L4, L5, L6 and L7. There was no significant difference in temperature variation between seasons in all structures ($p<0.05$) (Table 1).

Similarly, the relative humidity conditions of the eight structures were assessed for season one (wet) and two (dry). The highest average humidity was recorded in L0 (43.18±9.53) followed by structure L7 (42.89±7.37) %, while the lowest was recorded in structure L1 (33.26±7.29) %. Relative Humidity in the eight structures showed significant variations ($F_{0.05\ (7,\ 368)} = 12.78$, $p<0.0001$). Significant difference was noted between L0 and L3, L4, L5, L6 and L7. A similar trend was observed in season dry highest mean humidity was recorded in structure L7 (42.16±7.35) followed in structure L0 (29.34±0.85), while the lowest mean humidity was recorded in structure L1 (33.11±7.26) with a significant difference ($F_{0.05\ (7,\ 368)} = 10.77$, $p<0.0001$). L3 differed significantly from L4, L5, L6 and L7. L5 temperatures differed with that of structure L7. There was no significant difference in humidity variation between seasons in all structures ($p>0.05$).

Survival of silkworm larvae

The larval survival of silkworm followed a similar pattern among the two species tested. There was a high rate of the silkworm surviving to moulting to the third instar in all the structures for *B. mori.* During the wet season the highest survival was in L2 (76.73±4.20%), followed by L0 but was least in L5 with none surviving in L1, L6 and L7 beyond the fourth instar, which indicated a highly significant difference (Table 2). When the survival of Eri worms' larvae was considered in the tested structures for both seasons, the highest survival was in L0, followed by L3, but least in L5; however as in *B. mori* all the worms died in structures L1, L6 and L7 before attaining the fifth instar.

For *B. mori* during the dry season a higher survival was noted as compared with the wet season. Structure L0 (78.70±8.8%) recorded the highest survival rate which was highly significant compared with the other tested structures. The least survival percentage for the structures where the worms reached the fifth instar was in L5 (48.28±4.2%) which was similar to the wet season. Structurers L1, L6 and L7 recorded no survival worms beyond the third instars as all the worms died at the end of instar three and this was also observed for Eri worms. The Eri worm survival percentage followed a similar trend with the *B. mori*, L0 (80.10±3.7%), was the highest followed by L2 (80.07±3.7%), but the least was in L4 (56.77±9.9%.). However, in all the instars except in instar 2, survival percentage was found to significantly differ in all the structure for both seasons.

The duration of larvae of silkworm in the different structures

In L0, L2, L3, L4 and L5 structures, *B. mori* and Eri survived up to 5th instar taking different days (larval duration) to cocoon. *B. mori* larvae took 45.67 and 38.30 days during the wet season in structures L2 and L0 respectively (Table 3), while Eri took 39.33 days in structure L0 and L2 during the wet season and 30.33 days during the dry season for all the instars to cocoon in structure L0. The shortest duration was in the structures L5 (40.00±1.00 and 27.67±0.58) for *B. mori* in the wet and dry season respectively, which was found to be significantly different. Eri took the least days again in L5 at 34.33±0.58 days and 21.33±0.58 days during the wet season and dry season respectively and was significantly different among the structures. The larval duration was not significantly shorter in the dry season for *B. mori* (χ^2 = 4.1988, d.f.=4, p = 0.3798) and Eri, (χ^2 = 5.6277, d.f.=4, p= 0.2287) as compared to the wet season for the two species of silkworm.

Defective cocoons for *B. mori* in tested structures during wet and dry season

The number of defective cocoons for *B. mori* was established for the different structures per season. In wet season, majority of double defective cocoons in *B. mori* was recorded in structure L5 (37.5%) and Lowest in L4 (12.49%) in the structures where the defect was observed (Table 4) with a significant difference (χ^2 = 12.52, d.f.=3, p = 0.0058). The inside stained cocoon, highest percentage was in L2 (66.70%) and lowest in L4 (33.33%) with a significant difference (χ^2 = 178.9, d.f.=4, p = 0.0000). Outside stained percentage was high in L4 and L5 (29.00%) (χ^2 = 13.48, d.f.=4, p = 0.0000). Highest malformed cocoons were observed in L2 and L5, while flimsy cocoons were recorded in structures L0, L2 (19.98%) each and L5 (60.02%) recorded the most. There was a significant difference in percentage flimsy cocoons in the structures (χ^2 = 119.97, d.f.=4, p<0.0001). Pierced cocoons were found in structure L2, L3 and L4 which was significantly different (χ^2 = 65.41, d.f.=4, p = 0.0000) (Table 4). In the dry season the structures L2, L4 and L5 recorded the highest percentage of double defective cocoons (28.58%) (χ^2 = 212.5, d.f.=4, p<0.0000). Inside stained cocoons were more in L5 (75.02%) and lowest in L4 (28.98%) but none in L0, L2 and L3. Highest number of outside stained cocoons was recorded in L2. All structures during the dry season recorded malformed, but flimsy cocoons were recorded from all the structures except L3, while pierced cocoons were recorded only in structures L0 and L2.

Defective cocoons in Eri worm reared during the wet and dry season

In wet season, majority of double defective cocoons in Eri was recorded in structure L0 (75.0%) while during the dry season it was 66.7% (Table 5). Inside stained cocoon highest percentage was (33.33%) in L4 with a significant difference (χ^2 = 130.81, d.f.=4, p = 0.0000) in wet season as well as in dry season. Highest outside stained cocoons were recorded in season two in L5 (50.0%) and in season one it was in L2 and L3 (33.3%) (χ^2 = 62.60, d.f.=4, p = 0.0000). No malformed cocoons were observed in L0, L2, L3 and L5 for wet season while in the dry season L2 and L4 had 33.30% and 60% respectively. Flimsy cocoons were recorded in structures L3.

Table 1: Environmental conditions of experimental structures during wet (season 1) and dry (season 2)

	Season	L0	L1	L2	L3	L4	L5	L6	L7	F-Ratio	P-Value
Temperature (^{0}C)	Wet	22.73± 1.86^a	23.61± 1.25^{ab}	23.21± 1.71^b	25.00± 0.98^c	26.47± 0.67^d	27.45± 0.78^e	29.47± 0.86^f	31.63± 0.81^g	334.77	<0.0001
	Dry	22.86± 2.20^a	23.58± 1.29^{ab}	23.29± 1.65^b	25.02± 0.90^c	26.48± 0.61^d	27.36± 0.79^e	29.34± 0.86^f	31.52± 0.74^g	294.26	<0.0001
Humidity (%)	Wet	43.18± 9.53^a	33.26± 7.29^a	33.99± 5.05^{ab}	35.20± 6.48^{bc}	37.41± 7.81^{cd}	39.22± 7.87^{de}	40.80± 7.65^e	42.90± 7.37^e	12.78	<0.0001
	Dry	41.94± 10.78^{de}	33.11± 7.27^a	33.42± 5.40^a	34.51± 6.79^{ab}	36.87± 7.93^{bc}	38.82± 7.61^{cd}	40.24± 7.63^{de}	42.16± 7.38^e	10.77	<0.0001

Means denoted by a different letter in the same row are significantly different (0.05)

Table 2: Survival percentage of silkworm larvae under different structures during wet season

	Season		L0	L1	L2	L3	L4	L5	L6	L7	P (<0.05)
B. mori	Wet season	2nd	83.33±12.5	83.33±7.64	83.33±30.1	83.33±15.3	83.33±12.5	87.00±11.5	83.33±5.77	83.33±15.2	0.01
		3rd	82.37±8.61	63.33±4.51	77.03±3.96	78.03±2.51	74.30±12.6	66.87±11.7	73.70±9.86	62.90±4.19	0.04
		4th	79.23±13.6	0.00±0.00	77.03±12.9	78.0±7.35	70.30±6.04	66.10±8.11	0.00±0.00	0.00±0.00	0.00
		5th	76.70±8.78	0.00±0.00	76.73±4.81	76.00±8.54	60.00±6.25	56.67±4.16	0.00±0.00	0.00±0.00	0.00
	Dry season	2nd	83.33±12.6	82.80±2.6	83.33±15.3	82.37±8.61	82.33±12.6	87.00±11.5	83.33±11.6	83.33±12.6	0.01
		3rd	80.37±7.61	76.67±5.77	71.67±16.5	76.00±8.89	71.30±12.6	62.00±2.00	70.77±9.05	76.67±5.77	0.03
		4th	80.21±13.6	0.00±0.00	70.77±9.1	78.0±7.4	71.67±16.5	55.67±2.08	0.00±0.00	0.00±0.00	0.00
		5th	78.70±8.8	0.00±0.00	77.80±16.5	70.77±8.54	56.30±6.25	48.20±4.2	0.00±0.00	0.00±0.00	0.00
Eri worm	Wet season	2nd	82.73±6.1	83.33±12.6	82.67±16.3	88.03±9.1	82.80±2.6	81.07±7.7	83.33±11.6	83.33±15.3	0.99
		3rd	77.77±4.1	76.93±4.1	77.03±5.6	76.00±8.9	76.67±5.8	74.00±6.0	70.77±9.1	71.67±16.5	0.94
		4th	77.70±7.6	0.00±0.00	76.90±9.6	71.40±3.9	68.77±9.9	62.00±2.0	0.00±0.00	0.00±0.00	0.00
		5th	77.40±3.7	0.00±0.00	76.90±4.2	71.40±3.5	68.77±9.9	55.67±2.1	0.00±0.00	0.00±0.00	0.00
	Dry season	2nd	81.63±6.1	83.33±12.6	82.37±8.6	88.03±9.1	82.80±2.6	81.07±7.7	84.23±10.6	82.32±14.3	0.98
		3rd	78.75±4.1	76.93±4.1	76.00±8.9	76.00±8.9	76.67±5.8	75.00±6.0	70.76±8.1	71.57±15.4	0.93
		4th	76.40±7.6	0.00±0.00	77.70±7.6	76.67±5.8	67.77±9.9	62.00±2.0	0.00±0.00	0.00±0.00	0.00
		5th	80.10±3.7	0.00±0.00	80.07±3.7	78.00±9.9	56.77±9.9	59.70±1.1	00.00±0.0	0.00±0.0	0.00

Table 3: Duration (total number of days) taken by larvae of silkworm in the different structures

		L0	L1	L2	L3	L4	L5	L6	L7	F-Ratio	P-Value
B. mori	Wet	45.33±0.58	0.00±0.00	45.67±0.58	44.33±0.58	42.67±1.53	40.00±1.00	0.00±0.00	0.00±0.00	2108.70	0.00
	dry	38.33±0.58	0.00±0.00	37.33±0.58	36.00±0.00	32.33±0.58	27.67±0.58	0.00±0.00	0.00±0.00	5879.39	0.00
Eri	wet	39.33±0.58	0.00±0.00	39.33±0.58	36.33±0.58	35.33±0.58	34.33±0.58	0.00±0.00	0.00±0.00	5305.03	0.00
	dry	29.67±1.15	0.00±0.00	30.33±0.58	23.33±0.58	23.33±0.58	21.33±0.58	0.00±0.00	0.00±0.00	1666.57	0.00

Table 4: *B. mori* defective cocoons during wet and dry season

			structures			
	Season	**L0**	**L2**	**L3**	**L4**	**L5**
Double	Wet	25.00	25.00	0.00	12.50	37.50
	Dry	14.30	28.60	0.00	28.60	28.60
Inside stained	Wet	0.00	66.70	0.00	33.30	0.00
	Dry	0.00	0.00	0.00	25.00	75.00
Outside stained	Wet	14.30	14.30	14.30	28.60	28.60
	Dry	0.00	40.00	20.00	20.00	20.00
Malformed	Wet	0.00	40.00	0.00	20.00	40.0
	Dry	16.70	16.70	16.70	16.70	33.40
Flimsy	Wet	20.00	20.00	0.00	60.02	0.00
	Dry	25.00	25.00	0.00	25.00	25.00
Pierced	Wet	0.00	33.30	33.30	33.30	0.00
	Dry	50.00	50.00	0.00	0.00	0.00

Table 5: Eri defective cocoons for two seasons

Structure	**Season**	**L0**	**L2**	**L3**	**L4**	**L5**
Double%	Wet	75.00	33.30	0.00	0.00	0.00
	Dry	66.70	33.30	0.00	0.00	0.00
Inside stained%	Wet	0.00	0.00	0.00	33.30	100.00
	Dry	0.00	0.00	0.00	20.00	0.00
Outside stained%	Wet	25.00	33.30	33.30	0.00	0.00
	Dry	33.30	33.30	50.00	0.00	0.00
Malformed%	Wet	0.00	0.00	0.00	50.00	0.00
	dry	0.00	33.30	0.00	60.00	0.00
Flimsy%	wet	0.00	0.00	3.00	0.00	0.00
	dry	0.00	0.00	2.00	0.00	0.00
Pierced%	wet	0.00	33.30	0.00	0.00	0.00
	dry	0.00	0.00	0.00	0.00	0.00

Filament length for *B. mori* and Eri cocoon

For *B. mori* during the wet season, the longest Filament length was recorded from structure L2 (1377.80±150.17) m followed by structure L3 (1363.33±165.13) m while the shortest Filament length was recorded from structure L5 (1163.10±891.95) m (Table 5). There was a significant difference in filament lengths for *B. mori* recorded from the various structures in wet season (p = 0.0032). Significant difference was noted between structure L5 and all the other structures. During the dry season, the longest filament length for *B. mori* was recorded from structure L3 (1382.80±117.23) m followed by structure L2 (1377.80±150.17) m while the shortest filament length was recorded from structure L5 (1137,70±105.40) m. There was a significant difference in filament lengths for *B. mori* recorded from structures during dry season ($F_{0.05\ (4,\ 45)} = 6.028$, p= 0.0004).

The cocoon filament lengths for Eri silkworm in wet season, was longest in structure L2 (437.6±32.26) with shortest non-significant filament length recorded in structure L5 (397.61±46.82) m ($F_{0.05\ (4,\ 45)} = 1.53$, p= 0.2104). In dry season, the longest filament length was recorded from structure L2 (448.70±31.87) with shortest significant filament length recorded in L5 (376.70±40.42) m ($F_{0.05\ (4,\ 45)} = 4.92$, p= 0.0022). Significant difference was recorded between structures L0 and L4, L0 and L5, L2 and L4, L2 and L5 and also between L3 and L5. Seasons did not influence average filaments lengths resulting from the tested structures.

Table 5: Mean Filament length (m) for *B. mori* and Eri cocoon in different structures and seasons

Species	**Season**	**L0**	**L2**	**L3**	**L4**	**L5**	**F-ratio**	**p-value**
B. mori	Wet	1363.30± 165.13^{a}	1377.80± 150.17^{a}	1326.30± 117.20^{a}	1292.10± 84.12^{a}	1163.40± 91.95^{b}	4.64	0.003
	Dry	1361.50± 166.58^{a}	1377.80± 150.17^{a}	1382.80± 117.23^{a}	1288.50± 99.22^{a}	1137.70± 105.40^{b}	6.28	0.000
Eri	Wet	433.90± 55.65^{a}	437.60± 32.26a	427.50± 45.00^{a}	402.00± 54.56^{a}	397.60± 46.82^{a}	1.53	0.210
	Dry	445.70± 53.44^{a}	448.70± 31.87^{a}	436.30± 37.43^{a}	403.20± 54.52^{b}	376.70± 40.42^{b}	4.92	0.002

Means denoted by a different letter in the same row are significantly different (0.05)

Cocoon, pupa and Shell weight for *B. mori* and Eri during wet and dry season

Cocoon weight

During wet season, *B. mori*, cocoon weight was high in L2 (1.36±0.03) and L5 (1.36±0.05) (Table 6) and low in structure L0 (1.32±0.04) but did not differ among the structures (F= 1.08, p=0.3780). In dry season, *B. mori*, cocoon weight was high in structure L5 (1.78±4.30) and low in structure L4 (1.44±0.40) but did not differ among the structures (F= 0.77, p=0.5474). *B. mori* Cocoon weight was significantly high in dry season compared to season one for all the structures.

During the wet season, Eri cocoon weight was high in structure L0 (2.35±0.49) and low in structure L3 (1.82±0.35) (Table 4.10), with a significant difference in structures (F=

2.87, p=0.0333). In season two, cocoon weight was significantly high (F= 4.91, p=0.0024) in structure L3 (2.44±0.34) g and low in structure L4 (1.79±0.47) g. It was only in structure L0 where Eri cocoon weight was high in wet season in comparison to dry season.

Table 6: Mean Cocoon weight for *B. mori and* Eri cocoon in different structures and seasons

Species	Season	L0	L2	L3	L4	L5	F-ratio	p-value
B. mori	Wet	1.32± 0.04^{a}	1.36± 0.03^{a}	1.35± 0.06^{a}	1.33± 0.07^{a}	1.36± 0.05^{a}	1.08	0.378
	Dry	1.74± 0.16^{b}	1.65± 0.17^{b}	1.57± 0.13^{b}	1.44± 0.40^{b}	1.78± 4.30^{b}	0.77	0.5474
Eri	Wet	2.35± 0.50^{a}	1.90± 0.41^{a}	1.82± 0.35^{a*}	1.87± 0.39^{a}	2.03± 0.32^{a}	2.87	0.0333
	Dry	2.23± 0.26^{a}	2.23± 0.35^{a}	2.44± 0.34^{b*}	1.79± 0.47^{a}	2.06 0.27^{a}	4.91	0.0024

Means denoted by a different letter in the same row are significantly different (0.05)

Pupa weight for *B. mori* and Eri during wet and dry season

During wet season, *B. mori*, pupa weight was high in structure L5 (1.15±0.10) followed by L3 (1.11±0.27) and low in structure L0 (1.07±0.15) (Table 7) but did not differ significantly among the structures (F= 2.38, p=0.0653). In dry season, the *B. mori*, pupa weight was higher in structure L0 (1.44±0.14) and low in structure L4 (1.21±0.40) but did not differ significantly among the structures (F= 1.81, p=0.1429). *B. mori* cocoon weight was significantly high in dry season for L0 in comparison to wet season (p<0.05).

During wet season for Eri pupa weight, there was a higher weight in structure L0 (2.01±0.37) and low in structure L3 (1.55±0.37) with no significant difference in structures (F= 2.50, p=0.0554). In dry season, pupa weight was high in structure L3 (2.13±0.31) and low in structure L4 (1.59±0.42) with a significant difference (F= 3.95, p=0.0081). Significant difference was recorded between structures L0 and L4, L2 and L4, L3 and between L4 and L3 and L5. Structure L2 and L3 had high pupa weights in season two which was significantly different from that of season one (p<0.05).

Table 7: Pupa weight for *B. mori and* Eri worm

Species	Season	L0	L2	L3	L4	L5	F-ratio	p-value
B. mori	Wet	1.07±	1.09±	1.11±	1.10±	1.15±	2.38	0.0653
		0.15^{a*}	0.13^{a}	0.10^{a}	0.37^{a}	0.27^{a}		
	Dry	1.44±	1.39±	1.33±	1.21±	1.24±	1.81	0.1429
		0.14^{a*}	0.12^{a}	0.09^{a}	0.40^{a}	0.26^{a}		
Eri	Wet	2.01±	1.65±	1.55±	1.66±	1.78±	2.5	0.0554
		0.38^{a}	0.34^{a*}	0.37^{a*}	0.37^{a}	0.30^{a}		
	Dry	1.95±	2.00±	2.13±	1.59±	1.60±	3.95	0.0081
		0.26^{bc}	0.34^{bc*}	0.31^{c*}	0.43^{a}	0.27^{ab}		

Means denoted by a different letter in the same row are significantly different (0.05)

Shell weight for *B. mori* and Eri during dry and wet season

During season one (wet), *B. mori*, shell weight was high in structure L0 (0.23±0.07) and L3 (0.23±0.07) and lowest in L4 (0.20±0.07) (Table 8) but did not differ among the structures (F= 0.32, p=0.8645). In dry season, *B. mori*, shell weight was high in structure L3 (0.32±0.32) g and low in structure L5 (0.17±0.07) g but did not differ among the structures (F= 1.62, p=0.1862). *B. mori* shell weight was significantly high in dry season for L0 structure in comparison to wet season (p=0.0012).

During the wet season, Eri shell weight was high in structure L0 (0.30±0.13) and low in structure L4 (0.21±0.06) with no significant difference in structures (F= 1.20, p=0.3231). In the dry season, shell weight was high in structure L5 (0.44±0.62) and low in structure L4 (0.19±0.06) with no significant difference (F= 1.15, p=0.3450) similarly, shell weight did not differ among seasons (p<0.05).

Table 8: Shell weight for *B. mori* and Eri in wet and dry season

Species	Season	L0	L2	L3	L4	L5	F-ratio	p-value
B. mori	Wet	0.23±	0.22±	0.23±	0.20±	0.21±	0.32	0.8645
		0.07^{a}	0.06^{a}	0.08^{a}	0.07^{a}	0.07^{ab}		
	Dry	0.29±	0.23±	0.32±	0.20±	0.17±	1.62	0.1862
		0.06^{a}	0.07^{ab}	0.32^{a}	0.08^{ab}	0.08^{abc}		
Eri	Wet	0.30±	0.24±	0.26±	0.21±	0.23±	1.2	0.3231
		0.13^{a}	0.12^{ab}	0.10^{ab}	0.06^{ab}	0.07^{ab}		
	Dry	0.25±	0.21±	0.29±	0.19±	0.44±	1.15	0.3450
		0.05^{ab}	0.04^{abc}	0.06^{ab}	0.06^{abc}	0.62^{a}		

Means denoted by a different letter in the same row are significantly different (0.05)

Shell/pupa weight for *B. mori* and Eri

B. mori, shell/pupa weight was high in structure L2 (0.22±0.06) and low in structure L4 (0.16±0.07) in the wet season (Table 9) but did not differ among the structures (F= 1.78, p=0.1494). In dry season, *B. mori*, shell/pupa weight was high in L3 (0.24±0.22) and low in L5 (0.15±0.07) but did

not differ significantly among the structures (F= 0.62, p=0.6518).

Eri shell / pupa weight during the wet season was higher in L3 (0.19±0.16) and low in L4 (0.30±0.13) with no significant difference (F= 1.01, p=0.4142). In the dry season, the ratio was more in L5 (0.23±0.29) and low in L4 (0.11±0.03) with no significant difference, even among seasons (p<0.05) for the two species.

Table 9: Shell/pupa weight for *B. mori* and Eri

	Season	L0	L2	L3	L4	L5	F-Ratio	P-Value
B. mori	Wet	0.22± 0.06^{a}	0.17± 0.04^{ab}	0.16± 0.07^{ab}	0.16± 0.07^{ab}	0.16± 0.06^{ab}	1.78	0.15
	Dry	0.20± 0.04^{a}	0.16± 0.04^{ab}	0.24± 0.23^{a}	0.20± 0.20^{a}	0.15± 0.07^{ab}	0.62	0.65
Eri	Wet	0.14± 0.02^{ab}	0.14± 0.06^{ab}	0.19± 0.16^{a}	0.13± 0.04^{ab}	0.13± 0.04^{ab}	1.01	0.41
	Dry	0.13± 0.03^{ab}	0.11± 0.02^{ab}	0.14± 0.02^{ab}	0.11± 0.03^{ab}	0.23± 0.29^{a}	1.47	0.23

Means denoted by a different letter in the same row are significantly different (0.05)

Discussion

Larval Duration and Survival

The environmental conditions such as temperature and humidity highly determine the success of sericulture. These factors affect silkworm in all stages of development starting from hatching of silkworm eggs, which is the first and the most important foremost developmental event (Babu 2014; Srinath 2014; Kiplagat *et al.* 2022) [1, 7, 22] to cocoon formation.

Temperature and humidity directly affect the physiological functions of the worm (Thapa and Ghimire 2005; and Rahmathulla *et al.* 2012) [16]. The environmental conditions in the tested structures showed variation and was found to impact the survival percentage and larval duration in a similar pattern on the bivoltine and multivoltine silkworm in Uasin Gishu. Tazima, (1978) [24]; Oduor *et al.* (2016) [13] reported an optimal range of temperature of 21-27 °C with relative humidity of 70-85%. These conditions were recorded in structures labelled L0, L2 and L3 but an imbalance was noted in structures L1, L6 and L7, which resulted to the mortality of all the larvae without reaching the fourth instar. Further, the previous studies indicated that the mean performance of inbred silkworm lines under various conditions of temperature and humidity was significantly different from each other at various temperature and humidity exposures during 4th and 5th instars (Srinath 2014) [22].

The larval duration was found shorter in all the younger silkworms with a similar trend among the tested species, but longer in the older (5th instar), in all the structures, where survival was recorded. This could be due to the tolerance to humidity conditions coupled with their vigorous growth at this age than at near cocooning phase, as explained by Lertsatitthanakorn *et al.* (2006) [9] and Rahmathulla *et al.* (2012) [16].

A longer larval duration in structures having high temperatures and low humidity which could be due to low feeding and/or low food conversion efficiency rate as explained by Sharma and Kalita (2017) [20]. The multivoltine Eri worm larval duration was reported by Hailu (2016) [5] to be longer compared to the shorter duration of mulberry silkworm in structures with elevated temperature. Larval duration of between 21 days to 23 days for mulberry feeders and 23 to 24 days for multivoltine (23-24 days) indicating a slightly longer larval duration for Eri silkworm which is contrary to the current report. Pakhale *et al.* (2014) [14] study in India and Singh *et al.* (2002) [21] reported longer larval duration of 29±3 days, which they attributed to differences in temperature and humidity, which could explain the results reported in the current study. The ideal temperature range for Eri worm rearing is between 20 ºC and 35 ºC and an increase in temperature beyond that causes less spinning and mortality in larvae (Doloi *et al.* 2019) [3] the reason why Eri worms did not survive to 5th instar, in L6 and L7 could be due to these conditions.

Cocoon quality

Double cocoons occur when two silkworms spin the silk together, it occurs when temperature is high, in this research majority of double cocoons were observed in L5, this is due to high temperatures recorded in L5 which causes the mature worms to crowd thereby resulting to the spinning of cocoons together, this agrees with Taha *et al.* (2014) [23]. Inside stained cocoon occurs when the pupa dies inside the cocoon causing stain, it occurs when the temperatures become low causing longer larval duration making the worm to be susceptible to diseases thus death, this could explain the phenomenon of highest percentage recorded in L2, where the temperatures were the lowest compared to other structurers. Flimsy cocoons are cocoons with loose shell, of which majority was recorded in structures L4 and mostly this could be due to deformities of silkworm species. Pierced cocoons occur when the moth emerges from the cocoon and were found in structure L2, L3 and L4 because the structures were having relatively cooler conditions thereby a longer interval of the cocoon formation to the end of spinning. This resulted to some cocoons maturing earlier than others so that by the time of harvesting the other cocoons, were already getting to moth stage as explained by Lee (1999) [10].

Outside stained cocoons are cocoons with a spot on the shell caused by the absorption of intestinal fluid or urine of mature worms, this occurs when a mature worm crawls over already formed cocoon. In the present research it was found in all structures in wet season for *B. mori* whereas in Eri it was found in all structures apart from L4 and L5 which could be due to high temperatures in the structures and low humidity. High humidity at the time of spinning results to diuresis and cocoon staining. This explains why stained cocoons were found in structures with high humidity which are similar to what Ramachandra *et al.* (2001) [17] reported. The least defects in both seasons were in L3. This structure had a netting on the four flaps which allowed continuously opening of the polythene during the day and therefore provided aeration, which has been reported as one of the key requirements during spinning (Ramachandra *et al.* 2001) [17]. Defective

cocoons are poor quality cocoons since they are less reelable and the quality of silk filament produced is low.

Filament length for *B. mori* and Eri cocoon

Filament size deviation is an important commercial undesired characteristic of raw silk as a uniform filament size results to reduced breakages hence better weaving (Zulfigar *et al.* 2022). The research established that at high temperatures the filament length was low for each species in the respective structures. Which agrees with the research done by Lalitha *et al.* (2020) [8], who found out that when temperature is high it results to inferior quality cocoons and silk filament. The high temperature tends to shorten the larval duration resulting to less accumulation of silk thus shorter cocoon filament. When the environmental temperature is low, the larval duration is prolonged giving more time for silk accumulation thereby resulting to high filament length, this explains the fact that Eri cocoon length was highest in L2 for both seasons and *B. mori* longest length during the wet season. The present research established that Eri cocoons produce short filaments of silk while *B. mori* produce long silk filaments which agrees with previous research which showed that *B. mori* had an average filament length of 1028.26m though the present research recorded higher filament of more than 1100m in all the structures, partly because the silkworm species used in the study were hybrid. Eri highest filament length is 403.04m (Melesse *et al.* 2020) [11] which related closely to the 402m and 403 average length in L4 for dry season and wet season respectively recorded in this study.

Cocoon, pupa and shell weight for *B. mori* and Eri

The worms in the fifth instar ingest more than 88% of leaves and reaches its maximum weight within one or two days before they start spinning cocoons. In addition, they rapidly develop the silk gland which occupies 40% of their weight. Another research stated that adequate feeding of silkworm is important in cocoon production and further revealed that increasing frequency of feeding causes enhancement of cocoon shell weight, cocoon weight and shell ratio (Hosseini *et al.* 2008) [6], this strongly agrees with present research which found out that the above cocoon parameters were low in L5 the structure that had high mean temperature which affected growth performances of the larvae at later instars by affecting their physiological activities (Thapa and Ghimire 2005) [25]. When worms physiological activities are affected, feeding also reduces, and this was high in L0 and L2 whose temperature fluctuations were not extreme and rarely got to beyond 28 °C thus recording high cocoon weight. The average mean temperature in wet season was significantly lower compared to dry season which could be increasing physiological activities of the worms thus resulting to an increase in cocoon weight of *B. mori* which was significantly high during dry season in comparison to wet season for all the structures. Shell percentage calculated from the weight of cocoon gives the quantity of raw silk that can be reeled from a given quantity of fresh cocoon. Raw silk percentage is important in determining costs of raw silk as the 65-84% is the best according to ICIPE.

Conclusion

The mud-walled structure, a concrete walled house or green house with all flaps open during the day and closed at night ideal for silkworm rearing in where temperature and humidity fluctuate, further the duration of larvae in these structures was found to be equivalent to the ideal silkworm durations from other areas. Similarly, the silk cocoon quantity and quality were found to be best in green house with four flaps open (L3) and mud house (L0) for all the tested parameters, length, weight and lower pupa/shell ratio irrespective of the season.

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