Hatchability and Survival of silkworm *Bombyx mori* and Eri Worms under Controlled Environmental Conditions in Uasin Gishu County

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Abstract

Eri worm (Samia Cynthia racini), a domesticated non-mulberry silkworm is polyphagous and multivoltine, while mulberry silkworm (Bombyx mori) is bivoltine and feeds on mulberry leaves. Domesticated silkworms are highly sensitive to environmental fluctuations, making the adaptability to environmental conditions different from wild silkworm. In Uasin Gishu temperatures range between 8.4^{0}C and 27^{0}C which are not suitable for the hatching and rearing of silkworm. The experimental treatments were done in pre-constructed structures with the dimensions of equal dimensions (4mx4m and 3m height). Iron roofing with enclosed concrete walls (L0), iron roofing with enclosed timber walls (L1), iron roofing with mud walls (L2) and greenhouses with flaps designated L3 (green house with all flaps open,) 3 flaps open (L4), 2 flaps open (L5), 1 flap open (L6) and one completely enclosed (L7) were used to test hatchability, survival and durations. The green house flaps were fitted with a net gauze to protect entry of predators. The control condition was in a thermo scientific incubator (L8) at the laboratory, with 3 replicas under constant temperature and humidity. The temperature and relative humidity were recorded using hygrometer/thermometer, while the duration to every instar and survival was recorded. The mean room temperature ranged between 30.31^{0}C±1.7 in L6 to 23.85^{0}C±1.86 in L0, and mean relative humidity of 75%±0.00 (L7) and 29.93%±3.85 (L4) was statistically significant. The time to hatching was 3 days (L0) to 6 days (L1, L2). Percent hatching was 88.8% and 91.83% for B. mori and Eri respectively in L1 but lowest in L4. Larval duration (34 days) was longest for B. mori and 26 days for Eri. Larval survival percentage was highest in L2 (76.73±4.81) for B. mori and 77.40±3.67 for Eri (L0). Greenhouse with all flaps open and mud walled structure could provide suitable conditions for sericulture production in Uasin Gishu County.

Keywords: Eri worm, mulberry silkworms, hatchability, survival, duration

INTRODUCTION

Sericulture is the cultivation of silkworms to produce silk, by mature larva through the formation of a runny fluid before spinning a cocoon, the raw material for the production of silk (Donia, 2001). In Kenya both mulberry silkworm (*Bombyx mori* L.) and Castor plant Eri worm (*Samia Cynthia ricini*) have been introduced. Eri worms (*Samia Cynthia raccini*) is a fully domesticated non-mulberry silkworm (Sharma & Kalita, 2017), it is a polyphagous and multivoltine silkworm. Mulberry silkworm (*Bombyx mori*) is a domesticated species of silkworm bred for silk production feeding on mulberry leaves. The worms are the larval stages of *Bombyx mori* and *Samia cynthia racini* moths respectively. The adult female moths lay eggs which hatch after being fertilised by the male moth, the eggs hatch to larval stage. Understanding hatching behaviour is an important aspect in silkworm rearing for silk production (Xiang et al. 2018). The worms can be reared throughout the year depending on the presence of host plants. Silkworms are highly sensitive to environmental fluctuations due

to long periods of domestication. This makes the adaptability to environmental conditions of silkworm to be quite different from those of wild silkworm. According to Gong et al., (2020), adverse environmental conditions occur regularly and the way in which it can affect the development of the organism. Further, the regulation of these factors can improve silkworm crop (Rahmathulla, 2012). Controlled conditions are a way of optimizing temperature and humidity in regions like Uasin Gishu where temperatures range between 8.4^{o}C and 27 ^{o}C, which are not suitable for the hatching of silkworm. The research seeks to address this research gap by managing temperature and humidity through rearing silkworms in greenhouse and to compare with natural conditions for sustainable cocoon production in Uasin Gishu County.

MATERIALS AND METHODS

Hatching of Silkworms

The hatchability investigation was done using disease free layings of the bivoltine silkworm hybrid ICIPE 11 strain (Plate 1) and multivoltine *Samia Cynthia racini* (eri worm) obtained from the International Centre of Insect Physiology and Ecology (ICIPE). Two laying cards were obtained of each type and were transported to the laboratory during the morning cool hours to avoid drying out. In the laboratory the eggs laying were separated into groups of 250 eggs and each placed in a petri dish, which had been sterilized by wiping with cotton wool containing 2% formalin to eliminate any contamination.

Hatchability tests of silkworms under different conditions

Experimental hatching of silkworm (mulberry and castor plant silkworms) was conducted by picking each petri plate having 250 eggs in three replicates and placed under different conditions. The experimental treatments were done in pre-constructed structures with the dimensions of equal dimensions (4mx4m and 3m height each). Iron roofing with enclosed concrete walls (L0), iron roofing with enclosed timber walls (L1) to provide natural rearing conditions in Uasin Gishu, iron roofing with mud walls (L2) and greenhouses with flaps designated as L3 to represent green house with all flaps open, 3 flaps open (L4), 2 flaps open (L5), 1 flap open (L6) and one which is completely enclosed (L7) were used to test hatchability. The green house flaps were fitted with a net gauze to protect entry insects and other predators. The control condition was in a thermo scientific incubator (L8) at the biotechnology laboratory, with 3 replicas under constant temperature and humidity. Daily observation was made and the temperature and humidity for each condition was recorded until hatching. Hatching parameters like number hatched and hatching duration were recorded under the above different conditions for the silkworm hybrids. Counting was done at 6 hours interval and recorded. Counted larvae were gently brushed off with feathers into the rearing trays for further studies. From the recorded data, other parameters like day to day hatching average were extracted and hatching percentage were calculated.

Plate 1: The eggs of ICIPE II silkworm in the layings; The eggs of Eri silkworm

Data Analysis

All data generated from the experiment was entered into Excel spreadsheet for the purpose of management. Analysis was done using Statigraphic Centurion XVI and all values below 5% ($P \leq 0.05$) were designated as significant.

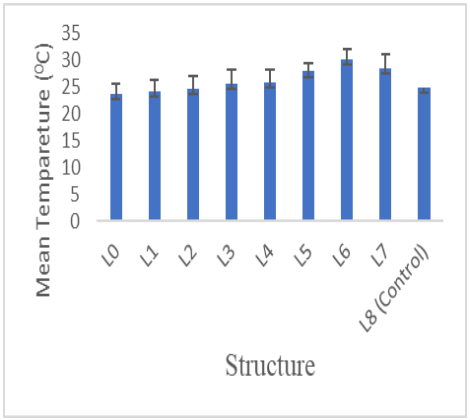
Survival and duration of silkworm larva under different conditions

To determine the suitable structure for the rearing of silkworm in Uasin Gishu, the pre-constructed structures used to investigate hatchability above were further used. Larva obtained after uniform hatching of eggs in incubator were introduced and reared. In each condition 200 hatched larvae in the 2nd instar with three replicates were reared in wooden trays (1mx 0.5m). The worms were fed twice per day at 8.00am and at 2.00pm with succulent freshly plucked leaves mulberry for *Bombyx mori* and castor leaves for Eri worms. The temperature and relative humidity were recorded in the morning, afternoon and evening, while at night the hygrometer/thermometer recorded the minimum and maximum conditions. The duration to every instar and survival of population under each condition was recorded daily until pupation and cocooning.

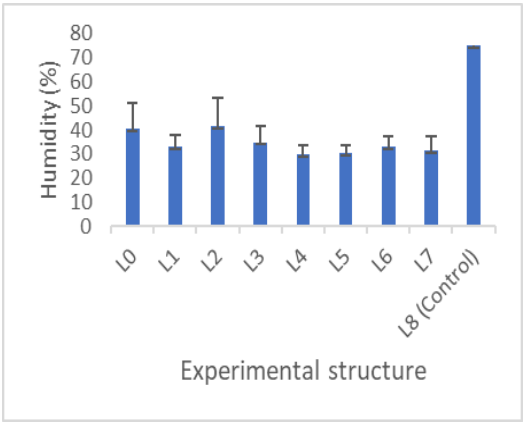
RESULTS

Environmental conditions of experimental structures

Temperatures conditions of eight structures were assessed during hatching of *B. Mori* and Eri silkworms' eggs and compared with control (L8). The Highest room mean temperature in ^{o}C was recorded in L6 (30.31±1.70), while the lowest mean temperatures were recorded in L0 (23.85±1.86) (Figure 1a). The temperatures in the eight structures tested for hatching of eggs of *B. Mori* and *Eri* silkworms showed variation ($F_{0.05\,(7,\,144)} = 20.94$, $p < 0.0001$). Significant difference was noted between L0 and L3, L4, L5, L6 and L7. When the mean temperature of the tested structures was compared with the ideal (L8) conditions, a significant difference was noted in L5 and L6 and L7, but was not significant with the temperature of other structures tested.



a



b

Figure 1: The temperature conditions inside the experimental structure during the incubation of *B. mori* and *Eri* eggs: 1b. Mean percent humidity conditions inside the structures during incubation of *B. mori* and *Eri* egg for hatching

Highest mean % humidity was recorded in the L8 (75.00±0.00). The tested L2 (41.56±11.59) and in the L0 (40.50±10.82) recorded the highest % humidity while L4 recorded the lowest (29.93±3.85) with a statistically significant difference ($F_{0.05\ (7,\ 144)} = 11.35$, $p < 0.0001$) (Fig. 1b). Humidity was significantly different in L0 and L1, L3, L4, L5, L6 and L7 structures.

Correlation between temperature and humidity inside the structures was established. Temperatures were negatively and significantly correlated with humidity in the L2 structure ($r=-0.5524$, $p=0.0142$) but insignificantly correlated in L0, L1, L3, L4, L5, L6 and L7 as illustrated in Table 1.

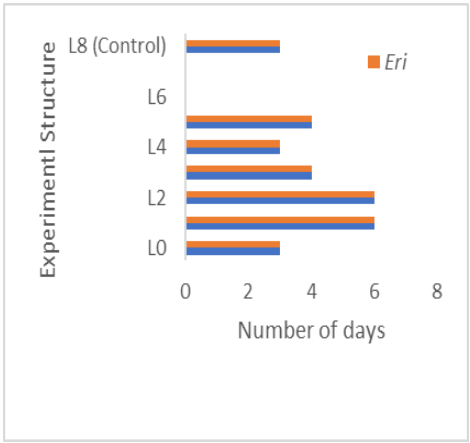
Table 1: Correlation between temperature and humidity inside the structures

Structure	Correlation (r)	p- value
L0	-0.23	0.34
L1	-0.42	0.071
L2	-0.55	0.014*
L3	-0.33	0.18
L4	-0.29	0.23
L5	-0.13	0.60
L6	-0.29	0.22
L7	-0.36	0.13
L8 (Control)	-	-

**Significant at 0.05*

Hatching duration of *B. mori* and *Eri* eggs in different structures

The time taken by *B. mori* eggs to complete hatching was between 3 days to 6 days. The longest duration was in L2 and L1 structures at six (6) days to hatch all eggs, four (4) days in L5 and L7, while in structures L1 and L4 they took only three (3) days for all eggs to complete hatching (Fig. 2a). The same trend was observed for *Eri* eggs. In the control (L8) hatching of *B. mori* and *Eri* eggs took an average of three (3) days to complete hatching. Further no eggs hatched when incubated under L6 and L7 structures.



a **b**

Figure 2: (a) Hatching duration of *B. mori* and *Eri* eggs, (b) The percentage of eggs hatched in the tested structures

Total number of *B. mori* and *Eri* eggs that hatched in the different structures was compared. In the control structure (L8), total number of eggs that hatched was 188 (94.0%) for *B. mori* and 193 (96.5%) for *Eri*. The highest mean number of *B. mori* eggs that hatched was in L0 (177.67±39.11) representing 88.8%, while L5 structure recorded the lowest number of eggs hatched (39.67±3.51) (18.67%) (Fig. 2b), with a significant difference ($F_{0.05\,(5,\,12)} = 499.48$). No hatching was recorded in structures L6 and L7.

For the *Eri*, highest total number of hatched eggs was recorded in structure L8 at 91.83% (183.67±5.13), followed by eggs incubated in L1 (179.00±6.42) (89.5%), but the lowest was recorded in L4 (32.22±4.51) (16.2%) followed by L5 (37.33±6.11) (18.7%) structures with a significant difference ($F_{0.05\,(5,\,12)} = 522.81$, $p < 0.0001$) as shown in Figure 2b. Mean significant difference in the total number of eggs hatched was between structures L0 and L1, L3, L4 and L5. There was also a significant difference in total number of eggs hatched between structures L1 and L2, L3, L4 and L5.

Pearson's Correlation for individual structure in relation to number of eggs hatched

Number of eggs hatched in structures L0 positively and significantly correlated with L8 (Control) as illustrated in Table 2. There was a positive correlation between number of eggs hatched in structures L0 and L2 (r=0.8581, p=0.3433), L0 and L3 (r=0.9143, p=0.2655) which was not significant, L0 and L5 (r=0.4678, p=0.6901), L1 and L4 (r=0.1037, p=0.9220), L3 and L5 (r=0.5766, p=0.6088), L3 and L8 (r=0.9286, p=0.2421) and between L5 and L8 (r=0.5000, p=0.6667). All the others showed a week negative correlation.

Table 2: Pearson's Correlation for individual structure in relation to number of eggs hatched

Structures	L1	L2	L3	L4	L5	L8 (control)
L0	-0.85	0.86	0.91	-0.99	0.47	0.99
	0.36	0.34	0.26	0.10	0.69	0.02*
L1		-0.45	-0.99	0.92	-0.87	-0.87
		0.70	0.09	0.25	0.33	0.33
L2			0.57	-0.76	-0.05	0.84
			0.61	0.45	0.97	0.37
L3				-0.97	0.79	0.93
				0.16	0.42	0.24
L4					-0.61	-0.99
					0.59	0.08
L5						0.50
						0.67

Correlation of conditions (temperatures and humidity) with duration to hatching

There was a positive insignificant correlation between eggs incubation period and the % humidity of the structures (r=0.2975, p=0.4369) but a negative correlation with temperature conditions (r=0.3349, 0.3784) (Table 3). Mean % humidity was negatively and insignificantly correlated with mean temperatures of the structures (r=0.2771, p=0.4704).

Table 3: Pearson correlation between mean eggs incubation period and the environmental condition of the structures

Eggs incubation period (days)	Humidity	Temperatures
Correlation	0.2975	-0.3349
p-Value	0.4369	0.3784
Correlation		0.2771
p-Value		0.4704

Survival of larvae of silkworm

The larval survival of silkworm followed a similar pattern among the two types tested. There was a high rate of the silkworm surviving to moult to the third instar in all the structures for *B. mori*. However, it is only in five structures where silkworm survived through to the fifth instar. In all the highest survival was in L3 (76.73±4.81%), followed by L0 (76.70±8.78%) but least in L5 (56.67±4.16%), with none surviving in L1, L6 and L7, which indicated a significant difference (Table 4). When the survival of Eri worms' larvae was considered in the tested structures the highest survival was in L0 (77.40±3.67%), followed by L3 (76.90±4.20%), but least in L5, however as in *B. mori* all the worms died in structures before attaining the fifth moult.

The duration of larvae of silkworm in the different structures

Larval duration, in days, was assessed in all structures. For *B. mori*, the 5th instar took the longest period (9 days) followed by 4th instar while 1st and the 2nd instar took the shortest duration with a significant difference ($F_{0.05\,(4,\,29)} = 44.54$, p= 0.0000). Similarly, for *Eri*, the longest larva duration was recorded at 5th instar with an average of 8.6±1.51 days while the least was recorded in 1st instar with an average of 2.50±0.92 days ($F_{0.05\,(4,\,26)} = 30.33$, p= 0.0000) as shown in Figure 3. In L0, L2, L3, L4, L5 structure, *B. mori* and *Eri* survived up to 5th instar taking different days (larva duration). The longest larva duration for *B. mori* was recorded in structure L0 (34 days), L2 (34 days) with L4 and L3 recording the lowest larval

duration with no significant difference (χ^2 = 1.649, d.f.=4, p = 0.7999). For *Eri*, the longest larva duration was recorded in structure L0 (26 days), L2 (26 days) and L4 (26 days) with lowest insignificant (χ^2 = 1.5462 d.f.=4, p= 0.8184) being recorded in structures L3 (20 days).

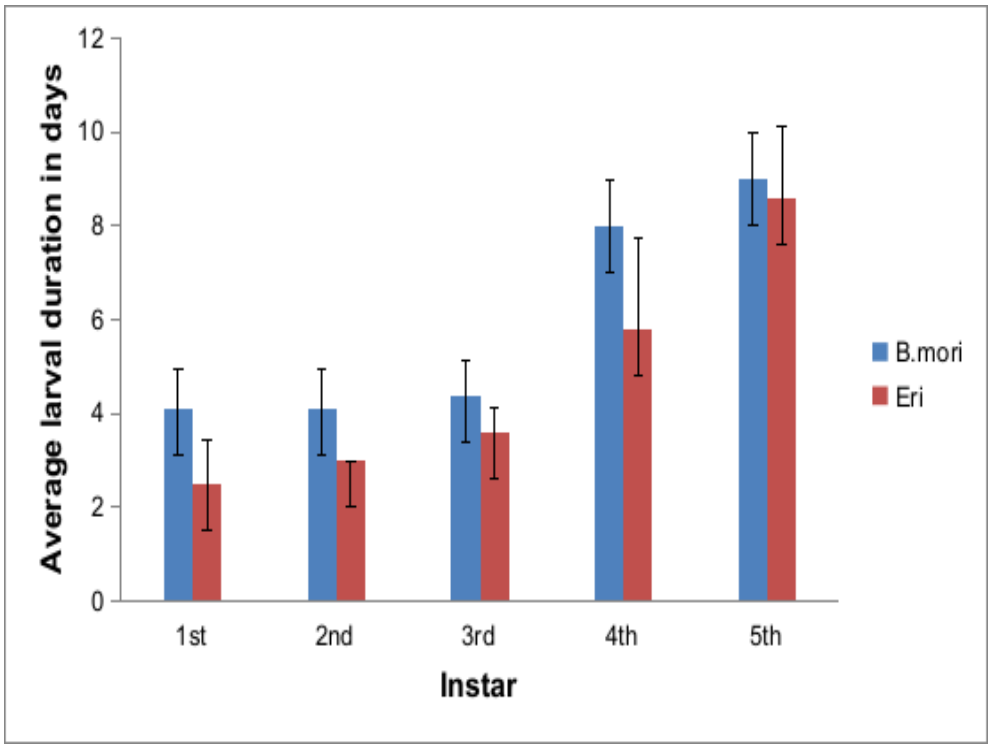


Figure 3: The larval duration of *B. mori* and *Eri* in the instars reared in the different structure conditions

Table 4. Survival percentage of silkworm larvae under different structures

Survival of *Bombyx mori in percent (%)*									
Instar	**L0**	**L1**	**L2**	**L3**	**L4**	**L5**	**L6**	**L7**	**P (<0.05)**
2nd	83.33±12.58	83.33±7.64	83.33±30.14	83.33±15.28	83.33±12.58	87.00±11.53	83.33±5.77	83.33±15.28	1.01
3rd	82.37±8.61	63.33±4.51	77.03±3.96	78.03±2.51	74.30±12.64	66.87±11.75	73.70±9.86	62.90±4.19	0.04
4th	79.23±13.61	0.00±0.00	77.03±12.95	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
***Eri* worm**									
	L0	**L1**	**L2**	**L3**	**L4**	**L5**	**L6**	**L7**	**P-Value**
2nd	82.73±6.07	83.33±12.58	82.67±16.29	88.03±9.10	82.80±2.59	81.07±7.70	83.33±11.55	83.33±15.28	0.99
3rd	77.77±4.09	76.93±4.11	77.03±5.56	76.00±8.89	76.67±5.77	74.00±6.00	70.77±9.05	71.67±16.50	0.94
4th	77.70±7.59	0.00±0.00	76.90±9.60	71.40±3.86	68.77±9.90	62.00±2.00	0.00±0.00	0.00±0.00	0.00
5th	77.40±3.67	0.00±0.00	76.90±4.20	71.40±3.53	68.77±9.90	55.67±2.08	0.00±0.00	0.00±0.00	0.00

DISCUSSION

The abiotic factors such as temperature and humidity determine to a large extent the success of the sericulture. These factors affect silkworm in all stages of development starting from the hatching of silkworm eggs, which is the first and the most important foremost developmental event (Shanthan, 2014; Srinath, 2014). The current research report indicated varying duration of incubation to hatching in the bivoltine mulberry and multivoltine Eri silkworm. These differences were reported in the tested structures which experienced varied temperature and humidity levels. Various authors have reported average of 10-12 days to hatching (Pakhale et al., 2014; Wankhade et al., 2014) under ideal conditions of temperature between 24ºC to 28ºC and humidity of 70-85%, these conditions prevailed in our experimental structures coded L0, L2 and L8 (control incubator), but in structures L4 and L5 there was no balance of humidity and temperature. Another observation in the present research was the duration to complete hatching for egg. A duration of up to six days were noted in structures L2 and L3, but lower in some structures with high temperature and low humidity. Datta (1992); Lertsatitthanakorn et al., 2006; Sharma & Kalita (2017) explained that the development of embryos in silkworm eggs attain uniformity, avoiding mixed age characteristics in balanced environmental conditions this could explain the current results observed in this experiment. It was observed that most of the hatching occurred on the first day of hatching and hatching was at the peak in the early hours of the day. Singh *et al*., (2002) reported that the hatching peak in *B. mori* was very close to dawn under alternating cycles of light and dark, which agrees with the results recorded in the current research. Temperatures above 30 degrees affect the metabolic function resulting in low hatching percentage (Rahmathulla, 2012). Similarly, temperature below 20 degrees causes inactivity in metabolic functions resulting in low hatching percentage and long duration taken to hatch. This could explain the low hatchability of eggs at the timber made structure, whereas the temperature of Uasin Gishu could be as low 15ºC during the night a condition which was obtaining during the duration of this research. This report is the first to indicate that hatching of silkworm can be done at the University of Eldoret under greenhouse and mud house structure and by extension the greater Uasin Gishu County, to moderate the diurnal and seasonal fluctuations in the outside environmental conditions.

Temperature and humidity combined largely determines the optimal growth of the silkworms and subsequent good-quality cocoons. This is because they directly affect the physiological functions of the worm (Thapa and Ghimire 2005; Rahmatulla, 2012 and Rahmathulla et al., 2012). The environmental conditions in the tested structures showed variation which was found to impact the survival percentage and larval duration in a similar pattern on the bivoltine and multivoltine silkworm in Uasin Gishu. An optimal range of temperature of 21–27 °C with relative humidity (RH) of 70–85% are ideal for silkworm (Tazima Y., 1978; Oduor *et al*., 2016). These conditions obtained in the structures which showed high survival rates, structures coded L0, L2, and L3, which showed a positive correlation with temperature and humidity, but poor in L4 and L5. The larval duration was found shorter in all the younger silkworms with a similar trend among the tested hybrids, but longer in the older (5^{th} instar), in all the structures, where survival was recorded. This phenomenon could be due to the levels of tolerance to humidity conditions and also due to their vigorous growth at this age than at near cocooning phase, agreeing with the results of Lertsatitthanakorn et al. (2006) and Rahmatulla et al. (2012). Similarly, there was longer larval duration in structures exhibiting high temperatures and low humidity this could be due to low rate of feeding and/or low food conversion efficiency as explained by Sharma & Kalita (2017). The multivoltine Eri worm larval duration was longer compared to the shorter duration of Mulberry silkworm in structures with elevated temperature. Abera, (2016) reported 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. But similar to Pakhale et al. (2014) study in

India. Singh et al. (2002) reported longer larval duration of 29+3 days, which they attributed to differences in temperature and humidity, therefore this could explain the results reported in the current study.

CONCLUSION

The hatching of silkworm eggs in Uasin Gishu can be in incubator or in mud walled structure or fully enclosed concrete structure.

Similarly, the rearing is best done in mud walled structure and greenhouse structure with four sited flaps opening during the day and enclosed at night.

ACKNOWLEDGEMENT

We Thank University of Eldoret Annual Research Grant for Funding this Research Ref. No. UoE/D/DVPRE/DRIV/NACO/074

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