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Artificial Hatching of Monovoltine Silkworm *Bombyx mori* L. Eggs by Using Hydrochloric Acid

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ABSTRACT

The present work was carried out to investigate artificial hatching of eggs of silkworm *Bombyx mori* L, Eggs were treated at 0, 12, 24 and 36 hours after oviposition by different dilutions of HCL ;1: 1.5, 1 : 2.0 and 1 : 2.5 (water to HCL in volume) at different exposure times; 5, 10 and 15 minutes. The eggs were incubated at 25°C aiming to rear the insect all over the year for different purposes. The results obtained may be summarized as follows. High hatchability percentages appeared in eggs treated after 24 hours from egg laying (more than 90% hatching). But when the time of immers on was prolonged to 36 hours, the percentage of hatching decreased. In the case of 0 and 12 hours, the percentage of hatching was so low. Hatchability was decreased when the concentration and the time of dipping of HCl increased, especially in the eggs with the age of 12 and 0 hour period, but hatchability increased in case of the eggs of 36 hours age when concentration and dipping time in HCl were increased. Larval mortality increased among larvae which were produced from eggs of 0 and 12 hour age in their second, third, fourth and fifth instars, but decreased in case of larvae which resulted from eggs treated at 24 and 36 hours age in larvae second, third, fourth and fifth instars.

Keywords: Artificial hatching – silkworm - hydrochloric acid HCL.

INTRODUCTION

Silkworm races reared under controlled conditions for a long time ago and adapted to temperate regions, which affected on hatchability and eggs fertility. On the other hand, the eggs fertility affected by mating duration and number of females per male (Abdelmegeed., 2015). The eggs stage in winters were be in a diapause status where diapause hormone secreted by the sub-esophageal ganglion (Kubota *et al*., 1979). Diapause occurred in insects when the environmental conditions are unsuitable. To enter into diapause, the embryo grows to a particular stage and then stops growing under natural conditions (Veda *et al*., 1997 and Singh *et al.,* 2013). Once the embryo enters to diapause status through the winter and not hatch except in the spring, the diapusing eggs are possible to stimulate by temperature, hot water, air pressure and chemical stimuli such as HCl treatment, nitric acid treatment, sulphuric acid treatment and Dimethyl Sulfoxide treatment to break the diapuse (Veda *et al.,* 1997 and Yamamoto *et al.,* 2013). HCl treatment is preferable method for breaking diapause in laboratory studies. This is because, HCl enter into eggs and affect embryonic protein synthesis system in yolk cells resulting in a complete embryogenesis (Park and Yoshitake, 1970 and Kai and Nishi, 1976). Although, the mechanism of HCL role at the molecular level is unknown, several hypotheses and reasons for its effect on the diapause status were studied (Yoshimi et al., 1990 and Tsurumaru et al., 2010).

This study was carried out to determine the best age of eggs after oviposition, the best dilution of HCl and the best dipping period of treatment by HCl to obtain healthy larvae which can be reared it in any time of year.

MATERIALS AND METHODS

Laboratory experiments were conducted on the monovoltine hybrid imported race of *B. mori* obtained from the Sericulture Research Department, Agricultural Research Centre. Ministry of Agriculture in form of eggs and reared under laboratory conditions of 25 ± 3 ^{0}C, 72 ± 2 % R.H. and 15:9 daily L:D. Just before starting experiments, the laboratory and implements were disinfected and precautions were taken against possibilities of certain epidemic diseases which may be attached mulberry leaves in the orchards. The rearing place was supplied with sufficient breeding frames (2 X 0.8 m , each). Soon after hatching, larvae were supplied with sufficient amount of mulberry leaves, *Morus alba* two times daily. The leaves were always cleaned from dust, and were given to the first and second larval instars as strips. Afterwards the diet was distributed in a manner that the larvae did not suffocate. At the end of each instar, bed cleaning nets were used to pick up the larvae and replaced their bed. The larvae were reared under standard rearing conditions (Krishnaswami, 1983). On 5th day of the 5th instar, full-grown larvae were collected manually and transferred to mountages for cocooning. The cocoons were spun within 72hr of mounting and seed cocoons were harvested on eighth day of spinning. Cocoons were preserved at 25±1°C and 75±5% RH. After accomplishment of emergence, moths were sexed and after copulation, the eggs laid during the first hour were pooled, sampled at indicated times.

HCl treatment

For artificial diapause broken by HCl, the newly deposited eggs (at 0 time age) and those of 12, 24 and 36 hours after oviposition were treated by HCl at dilutions of 1:1.5, 1:2 and 1:2.5 (water to HCL in volume) using the dipping methods.

The different groups contain three replicates/group, and each replicate contain 100 eggs which were dipped in different dilutions of HCl, for 5, 10 and 15 minutes, then the treated eggs were washed in running water and air-dried. All groups of the treated eggs were incubated at 25°C until hatching and the following data were recorded.

(1) Hatching %.
(2) Numbers of nonhatched treated eggs (those died during embryonic development).
(3) Mortality of larvae in the second, third, fourth and fifth instars.

RESULTS AND DISCUSION

Hatchability:

Data arranged in Table (1) showed that the hatchability of silkworm eggs has been significant affected by age of laid eggs, HCl dilutions and the dipping period. The highest hatchability percentage occurred in eggs of age 0 hour which were treated for 5minutes and with dilution of 1:1.5of HCl (30.7%), while the minimum hatchability in eggs of the same age were 0.0% when treated with 1:2.5 and 1:2 dilutions of HCl for 15minutes. The highly hatchability in eggs after 12 hours from oviposition were 71.4, 91.5 and 88.4% when treated for 5, 10 and 15 minutes with 1:2.5 dilution of HCL, respectively. The highly hatchability in eggs after 24 hour from oviposition occurred when treated for 10 minutes with 1:2.5, 1:2 and 1:1.5 dilutions of HCl (95.2, 94.1 and 95.7%, respectively). The highly hatchability in eggs after 36 hour from oviposition among eggs exposed for 15 minutes to 1:2.5, 1:2 and 1:1.5 dilutions of HCl (84.7, 84.6 and 62.5%, respectively). The maximum percentage of eggs which died during embryonic development after treated by HCl was found in eggs which treated after 0 hour oviposition for 15 minutes by high concentration of HCl. The minimum percentage of died eggs appear when eggs were treated after 36 hour from oviposition by lower concentration of HCl and for 5 minutes treatment.

Finally, it could be concluded that HCL in dilutions of 1:1.5, 1:2 and 1:2.5 were very effective for artificial diapauses termination specially on eggs of age of 24 hr with exposure time 10 and 15 minutes.

In this respect, Yamamoto *et al.,(*2013) found that the effect of DMSO was restricted within 24 hours after oviposition of diapaused eggs, and the critical period was slightly shorter than the effective period of the HCl treatment (20 hours after oviposition were treated with HCl (specific gravity 1.10) for 60 min at 25°C). Mizuta and Takahashi,(1958) found that the eggs in the hydrochloric acid (specific gravity, 1.100) for 12 hours at 5°C, more than 90% of them hatched. But, when the time of immersing was so long as 24 or 36 hours, the percentage of hatching decreased, and in the case of 36 hours, the percentage of hatching was low, being 60-71%.

Table 1 The percentage of hatchability and percentage of dead eggs after treatment with different dilution of HCl (water : HCl).

Age of treated eggs (in h)	Time of eggs dipping (min.)	Dilutions of HCl 1 : 2.5 HCl Hatchabi-lity %	1 : 2.5 HCl % dead eggs	1 :2 HCl Hatchabi-lity %	1 :2 HCl % dead eggs	1 : 1.5 HCl Hatchabi-lity %	1 : 1.5 HCl % dead eggs
0	5	7.9^{g} ±0.956	76.5^{b} ±2.384	13.6^{g} ±0.898	80.1^{b} ±3.120	30.7^{e} ±1.347	24.1^{b} ±1.721
	10	6.7^{g} ±0.648	79.0^{b} ±2.449	6.0^{h} ±0.509	84.4^{b} ±2.054	19.1^{f} ±1.470	45.3^{a} ±1.357
	15	0.0^{h} ±0.000	92.0^{a} ±1.519	0.0^{i} ±0.000	95.3^{a} ±2.007	14.7^{fg} ±1.608	47.9^{a} ±2.315
12	5	71.4^{d} ±1.430	17.4^{c} ±1.476	78.3^{cd} ±1.438	11.1^{c} ±1.592	32.2^{e} ±1.826	10.4^{c} ±1.347
	10	91.5^{ab} ±1.407	14.1^{c} ±1.532	74.8^{de} ±1.925	9.5^{c} ±1.256	83.5^{b} ±2.200	5.7^{d} ±0.873
	15	88.4^{bc} ±2.060	8.9^{d} ±1.635	80.1^{bc} ±1.621	9.4^{c} ±0.713	88.6^{b} ±1.721	6.4^{d} ±0.939
24	5	89.0^{bc} ±1.357	2.0^{e} ±0.793	79.8^{c} ±1.604	2.8^{d} ±0.659	50.8^{d} ±1.870	1.0^{e} ±0.478
	10	95.2^{a} ±1.779	1.5^{e} ±0.408	94.1^{a} ±1.630	2.0^{d} ±0.571	95.7^{a} ±1.189	1.1^{e} ±0.478
	15	91.5^{ab} ±1.562	2.1^{e} ±0.339	93.5^{a} ±1.430	2.3^{d} ±0.418	94.8^{a} ±1.630	1.0^{e} ±0.163
36	5	22.0^{f} ±1.395	1.5^{e} ±0.282	20.5^{f} ±1.349	1.2^{d} ±0.326	10.6^{g} ±0.941	1.0^{e} ±0.081
	10	64.3^{e} ±1.557	1.6^{e} ±0.163	71.1^{e} ±1.042	1.2^{d} ±0.163	30.9^{e} ±0.927	1.2^{e} ±0.163
	15	84.7^{c} ±1.158	1.2^{e} ±0.081	84.6^{b} ±1.070	1.4^{d} ±0.244	62.5^{c} ±1.476	0.8^{e} ±0.163

***:means with the same superscript in the same column were not significantly different ($p<0.05$)**
N.B. 300 egg/treatment

Larval mortality produced from eggs treated by HCl.

Among the resulted larvae from treated eggs, mortality increased when larvae advanced in instar. While mortality decreased with lower concentration of HCl and shorter dipping time. Data in Table(2) showed that the mortality rate of larvae in second, third, fourth and fifth instar produced from eggs treated by different concentrations of Hcl after oviposition. The maximum mortality rate occurred among larvae produced from eggs treated by HCl at 1:2.5 dilution for 10 minutes exposure. The mortality percentages were 7.3, 14.0, 38.0 and 60.0 % in second, third, fourth and fifth instars, respectively. In contrast, the minimum of mortality rate in larvae produced from eggs treated by

HCl at 1:1.5 dilution for 5 minutes exposure (2.7, 8.7, 20.7 and 29.7 % in second, third, fourth and fifth instar, respectively).

Data in Table(3) show that the mortality of larvae in their second, third, fourth and fifth instar produced from eggs treated by different dilutions of HCl after 12 hr of oviposition. The maximum mortality rate among larvae produced from eggs treated by HCl at 1:2.5 dilution for 15 minutes exposure, were 4.7, 5.7, 32.3 and 51.0 % in second, third, fourth and fifth instars, respectively. While, the minimum mortality rates occurred after treatment by HCl at 1:1.5 dilution for 5 minutes exposure, being 0.0, 0.7, 17.0 and 20.3 % in second, third, fourth and fifth larval instars, respectively.

Data in Table(4) showed the mortality of larvae in second, third, fourth and fifth instars produced from eggs treated with different dilutions of HCl after 24 h of oviposition. The maximum mortality rate occurred in larvae produced from eggs treated by HCl at 1:2.5 dilution for 15 minutes exposure, were 0.0, 8.7, 26.3 and 50.7 % in second, third, fourth and fifth instars, respectively. The minimum mortality rate occurred in larvae produced from eggs treated with HCl at 1:1.5 dilution for 5 minutes e, were 0.0, 0.0, 14.7 and 10.7 % in second, third, fourth and fifth instars, respectively.

Table 2 Mortality percentages in different larval instars resulted from eggs treated with different dilutions of HCl, immediately after oviposition.

HCl conc.	Time of eggs dipping	Larval mortality % Second	Third	Fourth	Fifth
1 : 2.5	5mn	5.0^{c} ±0.82	9.3^{bc} ±1.25	30.3^{b} ±1.69	50.7^{b} ±2.49
	10mn	7.3^{ab} ±1.25	14.0^{a} ±0.82	38.0^{a} ±1.63	60.0^{a} ±2.45
	15mn	-	-	-	-
1 : 2	5mn	4.3^{dc} ±0.47	8.0^{c} ±0.82	29.0^{bc} ±0.82	40.7^{c} ±2.49
	10mn	5.0^{c} ±0.82	11.3^{ab} ±0.47	25.3^{cd} ±0.47	40.7^{c} ±1.69
	15mn	-	-	-	-
1 : 1.5	5mn	2.7^{d} ±0.47	8.7^{bc} ±0.47	20.7^{e} ±0.94	29.7^{d} ±1.25
	10mn	5.3^{bc} ±0.47	9.0^{bc} ±0.82	23.3^{ed} ±1.25	40.3^{c} ±1.88
	15mn	7.7^{a} ±0.47	14.7^{a} ±1.25	30.7^{b} ±1.69	50.3^{b} ±0.47

*:means with the same superscript in the same column were not significantly different (p<0.05)

Table 3 Mortality percentages in different larval instars resulted from eggs treated with different dilutions of HCl, 12 h after oviposition.

HCl conc.	Time of eggs dipping	Larval mortality % Second	Third	Fourth	Fifth
1 : 2.5	5mn	0.0^{c} ±0.0	2.7^{cd} ±0.47	20.3^{cd} ±2.05	40.7^{b} ±2.49
	10mn	2.3^{b} ±0.47	4.0^{bc} ±0.82	30.3^{a} ±2.86	40.7^{b} ±2.49
	15mn	4.7^{a} ±0.47	5.7^{ab} ±0.47	32.3^{a} ±2.05	51.0^{a} ±2.94
1 : 2	5mn	0.0^{c} ±0.0	6.7^{a} ±0.47	20.3^{cd} ±0.47	30.3^{c} ±2.05
	10mn	0.0^{c} ±0.0	2.7^{cd} ±0.47	28.3^{ab} ±0.47	40.7^{b} ±1.69
	15mn	3.0^{b} ±0.82	1.3^{de} ±0.47	31.0^{a} ±0.82	40.7^{b} ±3.29
1 : 1.5	5mn	0.0^{c} ±0.0	0.7^{e} ±0.47	17.0^{d} ±0.82	20.3^{d} ±1.25
	10mn	0.0^{c} ±0.0	2.3^{cde} ±0.47	23.0^{bc} ±1.41	30.7^{c} ±1.69
	15mn	1.7^{b} ±0.047	2.7^{cd} ±0.47	29.3^{a} ±0.94	39.7^{b} ±1.25

*:means with the same superscript in the same column were not significantly different (p<0.05)

Table 4 Mortality percentages of different larval instars resulted from eggs treated with different dilutions of HCl, 24 h after oviposition.

HCl conc.	Time of eggs dipping	Larval mortality % Second	Third	Fourth	Fifth
1 : 2.5	5mn	0	2.3^{d} ±0.47	16.7^{cde} ±1.25	29.7^{b} ±1.25
	10mn	0	6.0^{b} ±0.82	22.3^{ab} ±2.05	30.7^{b} ±2.05
	15mn	0	8.7^{a} ±0.47	26.3^{a} ±1.25	50.7^{a} ±2.49
1 : 2	5mn	0	0.0^{e} ±0.0	15.7^{de} ±1.25	20.7^{c} ±1.69
	10mn	0	0.0^{e} ±0.0	20.7^{bcd} ±0.94	31.0^{b} ±2.94
	15mn	0	4.3^{c} ±0.47	22.7^{ab} ±0.47	30.3^{b} ±2.86
1 : 1.5	5mn	0	0.0^{e} ±0.0	14.7^{e} ±1.69	10.7^{d} ±0.94
	10mn	0	0.0^{e} ±0.0	18.3^{bcde} ±2.05	20.7^{c} ±0.94
	15mn	0	0.0^{e} ±0.0	21.3^{abc} ±1.88	20.7^{c} ±0.47

*:means with the same superscript in the same column were not significantly different (p<0.05)

Data represented in Table (5) showed the mortality of larvae in second, third, fourth and fifth instar produced from eggs treated with different dilutions of HCl after 36 h of oviposition. The maximum mortality rate occurred in larvae produced from eggs treated with HCl at 1:2.5 dilution for 15 minutes, were 0.0, 0.0, 17.3 and 30.3 % in the respective larval instars, respectively. The minimum mortality rate occurred in larvae produced from eggs treated with HCl at 1:1.5 dilution for 5 minutes, were

0.0, 0.0, 8.3 and 10.3 % in second, third, fourth and fifth instars, respectively.

Yamamoto *et al.,*(2013) found that the diapaused eggs which were not washed after the DMSO treatment, the hatching rate decreased. The same authors found that the prevention rates of diapauses after treatment with HCl and DMSO were approximately 90 and 78%, respectively.

Table 5 Mortality percentages of different larval instars resulted from eggs treated with different dilutions of HCl, 36 h after oviposition.

HCl conc.	Time of eggs dipping	Larval mortality %			
		Second	Third	Fourth	Fifth
1 : 2.5	5mn	0	0	$9.7^{c} \pm 0.47$	$10.7^{c} \pm 0.94$
	10mn	0	0	$15.7^{ab} \pm 2.49$	$21.0^{b} \pm 0.82$
	15mn	0	0	$17.3^{a} \pm 1.25$	$30.3^{a} \pm 2.05$
1 : 2	5mn	0	0	$9.0^{c} \pm 0.82$	$9.7^{c} \pm 1.25$
	10mn	0	0	$10.7^{bc} \pm 1.69$	$19.7^{b} \pm 2.05$
	15mn	0	0	$13.7^{abc} \pm 2.49$	$21.0^{b} \pm 1.88$
1 : 1.5	5mn	0	0	$8.3^{c} \pm 1.25$	$10.3^{c} \pm 1.25$
	10mn	0	0	$8.3^{c} \pm 0.94$	$9.7^{c} \pm 1.25$
	15mn	0	0	$11.3^{bc} \pm 2.05$	$10.7^{c} \pm 1.25$

*:means with the same superscript in the same column were not significantly different ($p<0.05$).

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التفقيس الصناعى لبيض دودة القز وحيدة الجيل باستخدام حمض الهيدروكلوريك

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استهدف البحث الحالى استخدام حامض الهيدروكلوريك لكسر طور السكون وتفقيس بيض دودة الحرير وحيدة الجيل صناعيا وذلك لتحديد أفضل تخفيفات الحامض والمدة المثلى لمعاملة البيض وعمر البيض المناسب لإجراء المعاملة من أجل تربية متواصلة للحشرة للأغراض المختلفة حيث استخدم الحامض بتخفيفات ١.٥ , ٢ , ٢.٥ بالحجم الى ١ حجم ماء ومعاملة البيض فى أعمار ٠ , ١٢ , ٢٤ , ٣٦ ساعة من الوضع ومدة تعريض البيض ٥ , ١٠ , ١٥ دقيقة. وكانت النتائج على النحو التالى: تم تسجيل أعلى نسبة فقس فى البيض (> ٩٠ %) المعامل بعد ٢٤ ساعة من وضع البيض وفترة تعريض من ١٠ الى ١٥ دقيقة وذلك مع كل من التخفيفات المستخدمة. تقل نسبة فقس البيض مع زيادة تركيز الحامض ومدة نقع البيض خاصة عند معاملة البيض عمر ٠ و١٢ ساعة, بينما تزداد نسبة الفقس فى حالة البيض عمر ٣٦ ساعة مع زيادة تركيز حمض الهيروكلوريك ومدة نقع البيض. تزداد نسبة موت اليرقات الناتجة من بيض معامل فى أعمار ٠ و ١٢ ساعة من الوضع وذلك فى الأعمار اليرقية الثانى والثالث والرابع والخامس بينما تقل نسبة الموت فى اليرقات الناتجة من بيض عمر ٢٤ و٣٦ ساعة من الوضع.