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METHOD OF COLD STORAGE AND ARTIFICIAL HATCHING OF AUTUMN
PRODUCED SILKWORM EGGS FOR SPRING REARING

by
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ABSTRACT

The F1 hybrid silkworm eggs of the locally developed variety (PAK 3 x PAK 4) laid at 22°C during autumn (November 14, 1990) were cold stored, 55 hours after ovi-position, at 5°C for 45 days and then at 2.5°C for 39 days. These were subsequently treated with Hcl solution of different concentrations for different durations for artificial hatching for spring rearing. Treatment with 10% Hcl solution for 5.5 minutes was found to be most suitable for obtaining the maximum useful hatchability of 97.6% under local conditions.

INTRODUCTION

The hibernating eggs of silkworm undergo a state of dormancy called diapause and do not hatch till the following spring after their exposure to a low temperature of about 5°C for several months to re-activate the embryo. In Pakistan, due to long unfavourable hot summer, the spring rearing is based on the hibernating eggs. The hibernating eggs can, since, produce only one generation in a year, it is, therefore, a limiting factor for making use of eggs laid in spring, for post spring and autumn rearing and those, laid in autumn, for spring rearing. This limitation has been overcome by developing methods for artificial hatching of eggs (Krishnaswami S. et. al. 1973). The most successful and rather the only method practiced at present is the acid treatment.

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In Japan, the acid treated eggs, produced during the current year are used for summer-autumn rearing and about 56% of the cocoon production comes from summer and autumn crops for which eggs are subjected to acid treatment to induce artificial hatching (Takeo Takami, 1967). In several other countries like Taiwan, Brazil, Korea and China, the eggs produced in autumn and treated for artificial hatching are reared in the following spring (Personal communication).

For immediate hatching, the time suitable for acid treatment is about 20-24 hours after oviposition, whereas for the postponement of hatching by more than 40 days, acid treatment is performed after cold storage of eggs (Tazima, 1978).

Krishnaswami (1973) reported that with the common acid treatment method, eggs can be made to hatch within 10 to 30 days by immediate acid treatment followed by cold storage for about 20 days and if hatching is to be delayed by more than 30 days, acid treatment can be carried out only after due chilling. In this method about 2 days old eggs are chilled for a minimum period of 40 days so as to awaken the embryo from diapause and when the embryo is awakened, the acid treatment takes place.

The method of common acid treatment is applicable for immediate hatching to take up rearing about 10 days after laying, but 30 days or so is the limit. Under this method, the possibility of artificial hatching of spring eggs for post spring rearing has already been investigated by the author by treating them, after 21 hours of oviposition, with 14.5 % HCl solution at 46°C for a period of 5.5 minutes to obtain 98% useful hatchability under local conditions (Muslim, 1977). For postponement of hatching by more than 40 days the cold treatment, as reported by Tazima Y. (1975), is

performed after cold storage of eggs. The best time for preservation of eggs in this way is 42 to 50 hours after oviposition and the eggs are transferred from 25°C to 5°C via an intermediate temperature of 15°C for 6 hours. High humidity of 75% to 80% is necessary during cold storage period.

Under the method of acid treatment after chilling, the eggs at a stage when they are strong enough to chilling are, in order to activate the hatching function, cold stored at a low temperature of 5°C and the insufficient hatching function is reinforced by dipping them in the acid solution as stated by Tajima Y. *et. al.* (1972). The hydrochloric acid, about 20% in concentration heated to 48°C, is used for dipping the eggs for 4 to 7 minutes depending on the silkworm varieties.

The shorter is the egg stage, better is the hatching and healthier are the hatched larvae. It is, therefore, desirable to use the eggs that were produced in the previous autumn for spring rearing and those produced in the spring of the current year for the summer-autumn rearing (Takeo Takami, 1967).

In Pakistan, the eggs produced in autumn may also be used for rearing in the following spring and which would, rather, be more convenient to handle owing to a shorter intervening period as compared to those produced in the previous spring and reared in the following spring. These eggs, however, shall have to be activated by acid treatment after due cold storage. The hatching of such eggs (Produced in Mid November) shall require to be delayed for about 23 days for spring rearing. The method of artificial hatching after ordinary chilling shall, therefore, be applicable for utilizing the

autumn eggs for spring rearing. This study was, hence, undertaken to have an understanding about the method of their cold storage and find the suitable strength of HCl and term of treatment to awaken the diapause embryo and effecting their artificial hatching in spring rearing season under local conditions.

MATERIAL AND METHODS

The parent silkworm eggs of local varieties PAK 3 and PAK 4, belonging to Japanese and Chinese breeds respectively, were incubated at 26^o-27^oC and reared at 22-25^oC during autumn, 1990 at Sericulture Research Laboratory, Lahore. The sexes of the two varieties were separated at pupal stage to avoid selfing. F1 hybrid eggs (PAK 3 x PAK 4) were produced by crossing the two varieties. The eggs were laid at 22^oC on November 14, 1990 and kept at 20-22^oC for 55 hours. The eggs were then refrigerated at 5^oC (via an intermediate temperature of 15-16^oC for 6 hours) for 45 days (November 17, 1990 to December 31, 1990) and then at 2.5^oC for 39 days (January 1, 1991 to February 8, 1991) till acid treatment. The humidity during the refrigeration remained about 80%. A control of nine egg layings in 3 replications of 3 layings each, was maintained by keeping at room temperature. The eggs of the control group were neither refrigerated nor treated with HCl.

The refrigerated eggs were taken out of the refrigerator on February 9, 1991 at 8.00 A.M. and kept at room temperature (15-16^oC) for 6 hours. The eggs were then dipped in 3% formaline solution for fifteen minutes, washed with tap water and dried. Fifty four egg layings were taken and divided into six groups of nine each. Nine layings of each group were

1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
4	4 (i)	15	6	75	20	2	-	-	97	95
	4 (ii)			80	10	5	-	-	95	90
	4 (iii)			65	30	-	1	-	98	95
	Average:-			73.3	20	2.3	0.3	-	96	93.3
5	5 (i)	14	6	65	20	5	3	-	93	95
	5 (ii)			75	15	5	2	-	97	90
	5 (iii)			70	20	5	4	-	99	90
	Average:-			70	18.3	5	3	-	96.3	91.6
6	6 (i)	10	30	10	35	25	1	2	73	60
	6 (ii)			15	30	15	-	2	62	45
	6 (iii)			10	45	20	2	-	77	65
	Average:-			8.3	36.6	20	1	2	70.6	56.6

No hatching was observed in the eggs of control group, which were neither refrigerated nor acid treated. The above result would reveal that the best useful hatchability (97.6%) was obtained in silkworm eggs produced in autumn (Mid November) and refrigerated, 55 hours of oviposition, at 5°C for 45 days and at 2.5°C for 39 days when treated with 18% Hcl solution at 48°C for 5.5 minutes. The results are in agreement with those reported by Tajima (1972). The hatchability obtained under the local conditions was, therefore, considered quite satisfactory.

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further sub-divided into three replications of three layings each. The various groups of eggs were treated with different concentrations of Hcl solution for different durations. The temperature of Hcl solution was kept constant at 49°C. The concentration of Hcl solution and term of treatment in respect of each group is given below:-

TREATMENT WITH HCL SOLUTION			
Group No.	Replication Number	Concentration of Hcl solution (%)	Term of treatment (Minutes)
1	1 (i)	20	5
	1 (ii)		
	1 (iii)		
2	2 (i)	18	5.5
	2 (ii)		
	2 (iii)		
3	3 (i)	16	6.0
	3 (ii)		
	3 (iii)		
4	4 (i)	15	6.0
	4 (ii)		
	4 (iii)		
5	5 (i)	14	6.0
	5 (ii)		
	5 (iii)		
6	6 (i)	10	30.0
	6 (ii)		
	6 (iii)		
7	7 (i)	Control. No treatment was given.	
	7 (ii)		
	7 (iii)		

The eggs were then washed thoroughly with running tap-water, air dried and kept at room temperature (15-16°C) till put to incubation on February 12, 1991.

The hatching started on February 22 and continued till February 25, 1991. The hatching data was recorded daily, replication-wise by ocular measures and averaged for each group. The useful hatchability was determined on the basis of the counts of the two major brushings and was expressed in percentage.

RESULTS AND DISCUSSION

The results of the experiment are given below in table 1.

TABLE 1

PRACTICAL AND USEFUL HATCHABILITY OF SILKWORM EGGS OF THE RACE (PAK 3 x PAK 4) F1 WHEN COLD STORED FOR 84 DAYS AFTER 55 HOURS OF OVIPOSITION AND TREATED WITH DIFFERENT CONCENTRATIONS OF HCL FOR DIFFERENT DURATIONS AT 43°C

Group No.	Rep. No.	Conc. of Hcl solution (%)	Duration of treatment (Min.)	Datewise Hatchability (%)					Total hatchability (%)	Useful hatchability (%)
				22	23	24	25	26		
1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.
1	1 (i)	20	5	80	15	2	1	-	98	95
	1 (ii)			75	20	3	-	-	98	95
	1 (iii)			85	10	1	2	-	98	95
	Average:-			90	15	2	1	-	98	95
2	2 (i)	18	5.5	90	8	1	-	-	99	98
	2 (ii)			85	45	-	-	-	100	100
	2 (iii)			70	25	3	-	-	98	95
	Average:-			81.6	16	3.3	-	-	99	97.6
3	3 (i)	15	6	85	10	-	2	-	97	95
	3 (ii)			60	35	1	-	-	96	95
	3 (iii)			80	10	5	3	-	98	90
	Average:-			75	19.3	2	1.7	-	97	93.3