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DEVELOPMENT OF SHORT-TERM EGG PRESERVATION SCHEDULE FOR TASAR SILKWORM, *ANTHERAEA MYLITTA* (DRURY)

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ABSTRACT

The commercially exploited ecoraces, Daba BiVoltine (DBV) and Daba TriVoltine (DTV) ecoraces of the Indian tropical Tasar silkworm, *Antheraea mylitta* Drury pupae remain in diapause from November to Mid-June and from January to May, respectively. During the termination of facultative diapause and the initiation of moth emergence period due to temperature fluctuations, DBV and DTV moths emerge erratically and lay eggs. The eggs laid by these erratically emerged moths get wasted as these do not coincide with the brushing schedule and there is no valid egg preservation technology developed so far. In this scenario, a short-term Tasar egg preservation schedule of egg preservation at 15°C for 15 days with 03 days of post preservation duration is reported in this paper. The hatching performance of treated and control eggs were found on par. The preservation technology can be effectively utilized for utilization of the eggs laid by erratically emerged moths during Grainage operations thereby improving the productivity in Tasar seed sector.

***Keywords*:** Tasar silkworm, erratic emergence, egg preservation, hatching, postponement.

Introduction

Indian tropical Tasar silkworm, *Antheraea mylitta* Drury undergoes facultative diapause during pupal stage, which occurs automatically until activation of dormant organisms (Denlinger, 2002; Ravindranath Singh *et al.,* 2014). The diapause period in the commercially exploited ecoraces, Daba Bivoltine (DBV) and Daba Trivoltine (DTV) is dependent on voltinism. DBV and DTV pupae remain in diapause from November to Mid-June (Almost 7-8 months) and from January to May (5-6 months), respectively. Since diapause termination and moth emergence patterns are greatly influenced by abiotic factors (Mishra *et al.,* 2011; Rahile *et al.,* 2015; Sailaja *et al.,* 2022), organizing a successful grainage is becoming a most difficult task. Processing of seed cocoons for production and supply of disease free layings to the stake holders, to meet the requirement of seed for tasar silk production is very important function of Basic Tasar Silkworm Seed Organization (BTSSO). In tasar seed sector, to supply disease free layings to the stakeholders, seed multiplication occurs in a multi-tier system through the seed organization, producing the total requirement of Nucleus seed and some portion of Basic seed to supply to the States. The States in turn produce remaining basic and commercial seed with support from private Graineurs to meet the ever-increasing demand of quality seed for higher silk production.

Owing to outdoor rearing, climate changes, pupal mortality during cocoon preservation period and irregular moth emergence during grainage operations, gap exists between demand and supply of quality seed. About 10-20% of valuable seed gets wasted because of prolonged and unsynchronized moth emergence (Ravindranath Singh *et al.,* 2014; Hasansab *et al.,* 2019; Sudhakara Rao *et al.,* 2019). In this context, short term tasar seed preservation schedule is required to minimize the wastage of tasar seed laid by unsynchronized or erratically emerged moths. The present study was aimed to develop a short-term egg preservation schedule, through which during Grainage operations erratically emerged moth laid eggs can be preserved and brushing of these eggs can be done along with the eggs laid by the uniformly emerged moths.

Materials and Methods

Tasar Daba TV and BV seed cocoons received from Basic Tasar Silkworm Seed Organization (BTSSO), Bilaspur, Chhattisgarh were kept at room temperature 25°C; 60±5% RH till moth emergence period at Silkworm Seed Technology Laboratory (SSTL), Bangalore. The moths were allowed to mate for a minimum of 8-9 hours and then depaired. The female moths were allowed to lay eggs in oviposition devices in dark conditions by maintaining temperature 25°C at 75±5% RH. Tasar eggs aged 48h, 60h and 72h were collected by considering the Zero-hour calculation of the age of the embryos (Vemananda Reddy *et al.,* 2003; Sailaja *et al.,* 2019).

Tasar silkworm eggs of different ages *viz.*, 48, 60 and 72h collected were washed as per the standard protocol and

each embryonic age eggs were preserved in cold storage at 5°C, 10°C, 15°C and 20°C temperatures by maintaining 80±5% RH in dark condition for 5, 10 15 and 20 days durations under Single Step Refrigeration Method (SSRM) to find the suitable temperature and cold tolerant embryonic age. After identification of cold sensitive embryonic ages as 60-72h. Two days laid tasar mixed eggs were collected and washed on the third day morning, where almost all tasar eggs cross 60h age were preserved at 15°C by maintaining continuous 80-85% RH for 15 days in dark condition. These eggs were released from Cold storage after preservation duration of 15 days to the incubation room, where 25°C of temperature and 80-85% RH were maintained continuously. After 3-4 days of post preservation/ progressive incubation duration, eggs started hatching. Hatching performance of the eggs preserved at different temperatures/durations was compared with the freshly laid eggs at room temperature (25 ± 5° C, 80% RH).

Results and Discussion

The eggs of DTV and DBV ecoraces of different ages and preservation duration showed different hatching performance at 5,10,15 and 20°C (Table 1). DTV and DBV eggs aged 48h preserved at 15°C for 10 days showed above 80% of hatching but declined hatching % was recorded after 10 days. 60h and 72h embryonic aged DTV and DBV eggs preserved at 15°C for 15 days showed hatching% more than 85% but 48, 60 and 72h embryonic aged eggs preserved at 15°C, for 20 days duration showed prior hatching on 17th day in cold storage and not completed the targeted duration of 20 days. The results clearly indicate that embryonic aged 60 and 72h Tasar eggs are sensitive and suitable for preservation at 15°C (Table 1) vis-a-vis the control batch, hatching performance of Trivoltine and Bivoltine eggs were recorded as 87 and 90 % respectively.

Standardization of Preservation schedule for mixed age eggs

In case of DTV and DBV, 85 and 80 percentage of hatching was recorded against 87% and 83% in the control, respectively and found no significant variation between preserved and control eggs hatching (Table 2). Thus, for the mixed lot of eggs (2 days laying), preservation for 15 days at 15°C and 80-85% RH with 2-3 days of incubation (total 18 days) has been found beneficial, without affecting the viability of the eggs.

Table 1 : Hatching performance of different age embryos preserved at 5°,10°,15°,20°C

Hatching Performance of Different Age Embryos at Various Temperatures						
Preservation (Days)	**Embryonic Age (DTV)**			**Embryonic Age (DBV)**		
	48h	**60h**	**72h**	**48h**	**60h**	**72h**
At 5°C						
5	78.11 (5P+5I)	80.72 (5P+4/5I)	82.32 (5P+4/5I)	80.35 (5P+5I)	82.42 (5P+4/5I)	84.39 (5P+4/5I)
10	65.77 (10P+5I)	74.73 (10P+3I)	78.85 (10P+2I)	68.16 (10P+5I)	77.36 (10P+3I)	80.26 (10P+2I)
15 & 20	0	0	0	0	0	0
S.Em±	0.339	0.277	0.234	0.099	0.125	0.104
CD @5%	1.231	0.700	0.722	0.306	0.384	0.320
At 10°C						
5	78.40 (5P+5I)	81.35 (5P+4/5I)	83.37 (5P+4/5I)	79.51 (5P+5I)	83.57 (5P+4/5I)	85.47 (5P+4/5I)
10	76.86 (10P+3I)	80.33 (10P+3/2I)	82.51 (10P+2I)	78.59 (10P+3I)	84.41 (10P+3/2I)	85.34 (10P+2I)
15	60.32 (15P+3I)	62.28 (15P+2I)	64.25 (15P+2I)	69.39 (15P+3I)	64.38 (15P+2I)	65.42 (15P+2I)
20	0	0	0	0	0	0
S.Em±	0.518	0.120	0.124	0.163	0.109	0.148
CD @5%	1.598	0.370	0.382	0.502	0.374	0.455
At 15°C						
5	83.51 (5P+5I)	84.45 (5P+4/5I)	84.49 (5P+4/5I)	84.37 (5P+5I)	86.37 (5P+4/5I)	88.41 (5P+4/5I)
10	84.30 (10P+3I)	86.22 (10P+3/2I)	85.32 (10P+2I)	86.36 (10P+3I)	87.35 (10P+3/2I)	90.29 (10P+2I)
15	75.35 (15P+3I)	85.41 (15P+2I)	85.24 (15P+2I)	74.40 (15P+3I)	86.35 (15P+2I)	88.38 (15P+2I)
20	69.26#	72.47#	74.49#	70.35#	74.36#	75.42#
S.Em±	0.152	0.144	0.164	0.150	0.149	0.171
CD @5%	0.469	0.442	0.506	0.462	0.459	0.527
At 20°C						
5	80.26 (5P+5I)	87.33 (5P+4/5I)	88.24 (5P+4/5I)	81.41 (5P+5I)	85.32 (5P+4/5I)	86.39 (5P+4/5I)
10	81.37#	88.45#	89.47#	82.50#	87.36#	88.41#
15	81.33#	87.46#	88.36#	81.56#	86.39#	57.54#
20	80.38#	86.32#	87.33#	82.53#	88.37#	89.37#
S.Em±	0.137	0.156	0.141	0.173	0.151	0.170
CD @5%	0.424	0.481	0.435	0.533	0.464	0.524
P=Preservation, I= Incubation, # Prior hatching						

Table 2 : Standardization of Preservation schedule for mixed age eggs

Hatching Performance of Mixed Age Eggs			
Races	**15 days Preservation** (15°C & 80-85% RH)	**Control** (~25°C & ~80% RH)	**p value** (t stat)
DTV	85±1.247 (@ 19 days)	87± 2.449	0.454
DBV	80±1.632 (@ 18 days)	83±3.681	0.323
15 days preservation and 2-3 days normal Incubation for treatment			

In *Bombyx mori* L, diapause exists only in bivoltine eggs and multivoltine eggs are non-diapause in nature. Anuradha *et al.* (2000) reported that multivoltine eggs in *B. mori* can undergo partial diapause and hatch over a period of 10-18 days. Sailaja *et al.,* (2019) found that through preservation of 36h muga embryonic age through temperature management, the non-diapause muga eggs chronological development (Circadian cycle of embryonic growth) can be slowed down. In present study, tasar eggs of 60-72h embryonic ages have been identified as suitable for preservation and can be preserved for 15 days with 02-03 days of progressive incubation. In this way eggs hatching can be postponed up to 18 days and also during 15 days of preservation, without affecting hatching performance eggs can be released on any day prior to the duration period. This schedule can be used to preserve the tasar eggs laid by the erratically emerged moths during Grainage operations for short duration and can be brushed along with the eggs laid by the moths emerged during peak Grainage operation.

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