DETERMINATION OF CRITICAL STORAGE PERIOD OF MASS REARED HOST EGGS PARASITIZED BY *TRICHOGRAMMA EVANESCENS* FOR EFFICIENT ADULT PARASITOID EMERGENCE

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ABSTRACT

This experiment was undertaken at IPM laboratory; Entomology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur to determine the critical storage period of mass reared host eggs parasitized by *Trichogramma evanescens* for efficient adult parasitoid emergence. Mass rearing of egg parasitoid, *Trichogramma evanescens* was done on *Sitotroga cerealella* (Olivier) eggs. Mass reared parasitized eggs of *Sitotroga cerealella* (Olivier) kept in desiccators at 3-4ºC and 75-85% relative humidity for 3 months. In this study number of *Trichogramma evanescens* adult emergence from 30 parasitized host eggs per test tube were observed, counted and recorded at 0,3,5,10,15,20,30,45,60,75,90 days after storage. In each case 5 test tubes were used as a replication and 3 generations of *Trichogramma evanescens* was observed. Results indicated that up to 20 days of storage more than 90% adult emergen ce from the parasitized eggs were observed and adult parasitoid emergence below 80% was observed after 45 days. It was also found that with the increase of storage period of parasitized egg, percent adult parasitoid emergence was gradually reduced. A negative correlation was observed with the storage period of parasitized egg and percent adult parasitoid emergence from the parasitized eggs. This suggests that for ensuring better performance of the parasitoid *Trichogramma evanescens* the parasitized egg should be stored not more than 45 days.

Keywords: Egg parasitoid, *Trichogramma evanescens*, adult parasitoid emergence, *Sitotroga cerealella*, mass rearing.

INTRODUCTION

Trichogramma (Hymenoptera: Trichogrammatidae) is a facultative gregarious (Rabinovich, 1971) egg parasitoid that often used in innundative biological control programs (Smith 1996) against a wide range of lepidopterous pest (Corrigan & Laing 1994; Li 1994). Many *Trichogramma* spp. are generalist egg parasitoid with broad host range including the Lepidoptera, Hymenoptera, Diptera, Coleoptera, Neuroptera and Megaloptera (Thomson & Stinner 1989; Li et al. 1994; Hoffmann et al. 1995). The success of this biological control relies not only on the time and the amount of natural enemies released, but also on their quality (emergence, longevity, fecundity, and searching capacity) (Bigler 1994; Cerutti and Bigler 1991; Dutton et al. 1996; Greenberg 1991; Van Lenteren 1991).

Storage of natural enemies assure their availability in sufficient number at the time of release. Therefore, the development of storage techniques for biological control agents is considered as an utmost importance to provide flexibility and efficiency in mass production, to synchronize a desired stage of development for peak release, and to make available standardized stocks for in use in research (Greenberg et al. 1996; Leopold 1998; Ravensberg 1992). Storage techniques must ensure the availability of quality natural enemies (Bigler 1994).

Although there are numerous investigations about storage of *Trichogramma* species (Greenberg et al. 1996; Jalai and Singh 1992; Krisnamoorthy and Mani 1999; Leopold 1998), it is important to study the amenability to cold storage for each particular species since not all of them are cold tolerable. For example, no adult emergence is observed when pupae of *Trichogrammatidae hactare* Nagarja are stored at 7ºC for 3 days (Krisnamoorthy and Mani 1999).

In Bangladesh mass rearing of *Trichogramma* generally been done on rice meal moth, *Corcyra ceyphalonica* but it is not cost effective and time saving. Recently, a cost effective mass rearing protocol of *Trichogramma evanescens* (Oliver) on angoumois grain moth, *Sitotroga cerealella* eggs was developed (Alam et al. 2009a).

Trichogramma evanescens (Oliver) is the most commonly used insect antagonist in Bangladesh. Alam et al. (2009b) carried out some experimental releases of *Trichogramma evanescens* against *Leucinodes orbonalis* Guen. (Lepidoptera: Pyralidae) on eggplant, leaf eating caterpillars such as diamond back moth and *Spodoptera litura* infesting cabbage (Alam et al. 2009c) and *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) attacking chickpea (Sarkar et al. 2009) with promising results. Because of the wide host range *T. evanescens* and the results obtained in the previous studies mentioned above, this species could be considered as an effective biological control agent of many agricultural insect pests. In Bangladesh, no storage technique has yet been developed for this species. Therefore, the present study was undertaken to determine the critical storage period of mass reared host eggs parasitized by *Trichogramma evanescens* for their efficient emergence.

RESULTS AND DISCUSSION

It is observed from Table 1 that, 5 days after storage more than 99% adult emergence from the parasitized eggs was evident and up to 20 days it was found above 90%. Then adult parasitoid emergences were gradually decreased. It is evident that adult parasitoid emergence below 80% was observed after 45 days. Lowest percent adult parasitiod emergence from the parasitized eggs was observed after 90 days of storage. So, we can conclude that with the increase of storage period of parasitized egg, percent adult emergence of *Trichogramma evanescens* was gradually reduced. A negative correlation was observed with the increase of storage period of parasitized egg and percent adult parasitiod emergence from the parasitized eggs (Fig. 1).

Table 1. Effect of different storage period of parasitized egg on adult emergence of *Trichogramma evanescens*

Cold storage period day(s)	Adult emergence from parasitized 30 egg *Mean ± SE	% Adult emergence of *Trichogramma evanescens* Mean ± SE
0	29.73±0.07	99.11±0.22
3	29.60±0.12	98.66±0.38
5	29.70±0.06	99.00±0.19
10	28.93±0.29	96.45±0.96
15	27.93±0.68	93.11±2.25
20	27.07±0.87	90.22±2.88
30	26.60±0.76	88.67±2.52
45	24.67±0.44	82.22±1.45
60	22.07±0.44	73.56±1.45
75	16.93±0.20	56.44±0.67
90	15.80±0.23	52.67±0.77

* mean of 3 generation

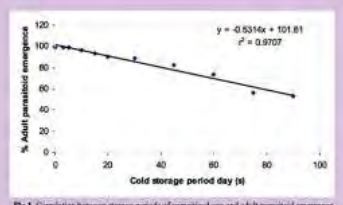

Fig.1. Correlation between storage periods of parasitized egg and adult parasitoid emergence

This study showed that *T. evanescens* parasitized eggs are amenable to cold storage. However, cold storage affected the adult emergence. Prolonged low temperature had a detrimental effect on the survival of *T. evanescens* parasitized eggs (Table 1). A reduction in the emergence due to the cold storage was also seen in other *Trichogramma* spp. (Ventura Garcia et al. 2002; Tezze and Botto 2004). Emergence of *T. evanescens* after 45 days at 4 ºC was more or less similar to those obtained for *T. nerudai* stored for 50 days (Tezze and Botto 2004) and for *T. cordubensis* stored for 60 days at 3ºC (Ventura Garcia et al. 2002). The adult emergence was found to be above 80% from control group up to 45 days of storage, it could be possible to cold storing parasitoids until 45 days without a significant effect on their quality. This is in accordance with Jalai and Singh (1992), Ventura Garcia et al. (2002) & Tezze and Botto (2004), who reported that *Trichogramma* spp. were not tolerant to cold storage up to 50-60 days, because after this period they do not emerge or emerge with low viability.

Storage of *T. evanescens* at low temperature could be useful for inundative biological control strategies as well as in inoculative release programs. Regarding the development of storage techniques for mass rearing of native species is important since releasing these species rather than the introduced ones, because it has the advantage that the *T. evanescens* are already adapted to the local environmental condition.

From this study it is recommended that for ensuring better performance of the parasitoid *Trichogramma evanescens* the parasitized egg should be stored for not more than 45 days.

MATERIALS AND METHODS

Mass rearing of parasitoids

Five kg wheat poured into boiled water for 2-3 minutes. Then the heat treated wheat of 2.5 kg was kept in steel trays (50 cm x 40 cm), each tray containing 2.5 kg and 1 gm of *Sitotroga cerealella* eggs were placed on the wheat and left for 5-6 days in undisturbed condition. Then plain water was sprayed over the infested wheat using hand sprayer and mixed properly with gentle stirring. After 22-25 days, the infested wheat with *S. cerealella* larvae, were put in mass rearing chamber (75 cm x 60 cm x 150 cm) for adult emergence. Thousands of *S. cerealella* adults were collected from the mass rearing chamber and kept in a glass cylinder (13 cm x 23 cm) and its mouth was covered by 32 mesh net. Adults were left in the cylinder for a day to allow mating and subsequent egg laying within the wall of glass cylinder. On the following day the eggs on the wall of the cylinder were brushed carefully and sieved by a sieve (30 mesh net) to separate eggs. Sieved eggs were then cleaned by using an exhaust fan and removed the dead adults, their body parts and scales to get the fresh eggs.

Five gm fresh eggs of *S. cerealella* were then placed in a long moist glass cylinder (glass cylinders (9 cm x 21.5 cm) were moistened by keeping them inside freezer for few minutes) and the eggs were spread over by rolling the cylinder gently. One gm parasitized eggs of were placed inside a vial (2 cm x 6 cm) were then kept inside the glass cylinder and its mouth was covered by white clothes. Parasitization took place under standard rearing conditions (25.0±2.0 ºC with of RH 80±5% and continuous inflorescent light) until they reached at pupal stage. Within 9-11 days parasitization of almost all the *S. cerealella* eggs were taken place.

Experimental methods

Mass reared parasitized host eggs of *Sitotroga cerealella* (Olivier) described above were randomly allocated to each treatment. Each treatment consisted of five test tubes (2.5 cm x 15 cm) with 30 parasitized host eggs per tube and stored for 3, 5, 10, 15, 20, 30, 45, 60, 75, 90 days in desiccators (40 cm x 30 cm). Now the desiccator was placed in a refrigerator maintained full darkness at 4±1 ºC & 80±5% RH. Similar temperatures (around 4 ºC) were maintained in the cold storage for other *Trichogramma* spp. as reported by Greenberg et al. 1996; Jalai and Singh 1992. In each case 5 test tubes were used as a replication and observed for 3 generations of *Trichogramma evanescens*.

A control group was kept at standard rearing conditions. Once the storage period was over, the treated groups were transferred to the rearing chamber and maintained at standard rearing conditions. Finally, the number of emerged adult *T. evanescens* from host eggs was observed under a binocular microscope, counted and recorded according to the morphological standard of Wang et al. (1981).

Statistical analysis

The results were analyzed using MS Excel software; data in the figures and table are presented as mean ± standard error.

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