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Effects of low temperature storage on parasitizing efficiency of egg parasitoid, *Trichogramma japonicum* (Ashmead)

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Abstract
The pupae of *Trichogramma japonicum* were stored at five cold storage temperatures (5, 7.5, 10, 12.5 and 15 °C) for nine storage durations (5, 10, 15, 20, 25, 30, 35, 40 and 45 days) in incubators. The adult emergence (%) from stored pupae and host (*Corcyra cephalonica* Stainton) egg parasitization by the emerged adult parasitoid were studied under standard laboratory conditions. The adult emergence of 93.07% was found to be the maximum at 7.5 °C stored for 5 days. The emergence gradually decreased with the increase in duration. After 30 and 45 days of storage the emergences were reduced to 76.48% and 52.50%, respectively. The parasitization of 91.56% was found to be the maximum at 7.5 °C stored for 5 days. The parasitization rates were gradually reduced with the increase in storage duration and reached to 71.74% and 54.58% at 30 and 45 days after storage, respectively. The next best temperature was 10 °C followed by 5 °C. The higher temperatures *viz*., 12.5 °C and 15 °C were found unsuitable for the storage of *Trichogramma japonicum* pupae.

Keywords: cold storage of pupae, *Trichogramma japonicum*, adult emergence, host egg parasitization

Introduction
Chemical pesticides are widely used in agriculture because of their immediate and tangible effect. However, due to indiscriminate use of pesticides, there is enormous harm to both living beings and the environment (Gupta and Dikshit, 2010) [3]. So, an environmentally friendly alternative to solve pressing needs of pest management is 'Biological Control' (Ghosh and Ballal, 2018) [2]. Trichogrammatids are a group of minute wasps of great importance to biological control. This group of insects can be easily reared in laboratories and have a great parasitizing potential against the egg of the target hosts (Nadeem *et al.*, 2010) [7]. Thus they were widely utilized as biocontrol agents. *Trichogramma japonicum* A shmead (Hymenoptera: Trichogrammatidae) is an important egg parasitoid used in integrated pest management of lepidopteran borers of sugarcane, cotton, maize, paddy etc (Jalali and Singh, 1992; Bhargavi and Naik, 2015) [4, 1]. The storage of parasitoid pupae retards their development so as to harmonize their emergence with presence of vulnerable stages of the host in the field. Thus, low temperature storage is considered as an indispensable prerequisite in biological control (Kumar *et al*., 2005) [6]. Nadeem *et al.* (2010) [7] suggested that the developmental period of parasitoids is directly influenced by temperature. Hence, the present study was conducted to estimate the effects of storing the pupae of egg parasitoid, *Trichogramma japonicum* at low temperatures on its emergence and parasitizing potential.

Materials and Methods
The studies were conducted at Biocontrol Laboratory, Department of Entomology, OUAT, during 2018-20 to ascertain the effects of low temperature storage of pupae of the egg parasitoid, *Trichogramma japonicum* on its parasitizing efficacy. UV light treated *Corcyra* eggs (200 each) were glued on paper card strips (5cm X 2cm). Each of these egg cards were exposed to ten pairs (selected in mating condition) of 12 h old *T. japonicum* adults inside a specimen tube for 24 hours. The parasitized egg cards were taken out of the specimen tube and kept under standard laboratory condition *i.e.*, 28±2 °C temperature and 75±5% RH for 5-6 days. The parasitoid developed inside the host eggs, which turned black upon pupation of the parasitoid larvae. The parasitized egg cards were stored at five constant temperatures *viz*., 5.0, 7.5, 10.0, 12.5 and 15.0 °C each for 5, 10, 15, 20, 25, 30, 35, 40 and 45 days inside the BOD incubator with complete darkness.

The parasitized egg cards were removed from the BOD incubator after a specific storage period and kept at standard laboratory condition. The adult parasitoids emerged and were offered with fresh UV light treated *Corcyra* egg cards for parasitization. Observations on the number of adults emerged and number of eggs parasitized were recorded daily under stereozoom microscope. The per cent emergence and per cent parasitization at each temperature and duration combination were calculated using the following formulae.

$$\text{Emergence percentage (EP)} = \frac{\text{No.of adult emerged}}{\text{No.of black (parasitized) host eggs on card}} \times 100$$

$$\text{Parasitization percentage (PP)} = \frac{\text{No.of black (parasitized) host eggs on card}}{\text{Total no.of host eggs on card}} \times 100$$

Results and Discussions

The data shown in Table-1 revealed that the maximum per cent adult emergence was recorded from pupae stored at 7.5 °C for 5 days (93.07%) followed by 10 days (91.93%) which were at par. These adult emergence data were followed by pupae stored at 10 °C for 5 days (90.13%), at 5 °C for 5 days (89.67%) and at 10 °C for 10 days (89.53%). The difference among these three treatments were statistically non significant. There was gradual reduction in the emergence percentage for each temperature with increase in storage period. The per cent emergence was reduced to 76.48% and 52.50% after 30 and 45 days of storage period, respectively at 7.5 °C. The data on adult emergence at 10 °C were 71.65% at 30 days and 51.27% at 45 days and at 5 °C were 70.21% at 30 days and 50.28% at 45 days. The higher temperatures *viz*., 12.5 °C and 15 °C showed comparatively lesser per cent adult emergence. Besides, the adults emerged inside the BOD incubator when stored beyond 40 days at 12.5 °C and beyond 20 days at15 °C.

Table 1: Effect of storage of *T. japonicum* pupae on adult emergence (%)

Duration	Adult emergence (%)(in different storage temperature)					
	5 °C	**7.5 °C**	**10 °C**	**12.5 °C**	**15 °C**	**Mean**
5Days	89.67 (71.29)	93.07 (74.81)	90.13 (71.73)	82.64 (65.37)	60.23 (50.89)	83.15
10Days	86.69 (68.62)	91.93 (73.51)	89.53 (71.15)	76.50 (60.99)	49.66 (44.79)	78.86
15Days	81.86 (64.79)	87.52 (69.31)	82.63 (65.37)	65.70 (54.14)	42.89 (40.90)	72.12
20Days	79.67 (63.19)	84.91 (67.17)	79.77 (63.29)	45.89 (42.63)	37.78 (37.92)	65.61
25Days	74.63 (59.75)	81.54 (64.55)	76.45 (60.97)	33.65 (35.45)	0.00 (0.00)	53.25
30Days	70.21 (56.90)	76.48 (60.99)	71.65 (57.83)	28.23 (32.08)	0.00 (0.00)	49.31
35Days	61.35 (51.55)	66.93 (54.91)	64.04 (53.16)	19.93 (26.50)	0.00 (0.00)	42.45
40Days	53.52 (47.01)	58.08 (49.63)	56.26 (48.60)	8.34 (16.76)	0.00 (0.00)	35.24
45Days	50.28 (45.15)	52.50 (46.43)	51.27 (45.71)	0.00 (0.00)	0.00 (0.00)	30.81
Mean	71.99	77.00	73.53	40.10	21.17	
	SE(m)±			CD(0.05)		
Temperature	0.264			0.742		
Duration	0.354			0.995		
T X D	0.791			2.225		

*Figures in the parentheses are angular transformed values

The data shown above revealed that the trichocards parasitized with *T. japonicum*, upon blackening, could be effectively stored up to 30 days at 7.5 °C. That was followed by storage at 10 °C and 5 °C up to 30 days. The findings of Ghosh and Ballal (2018) [2] revealed that *T. japonicum* can be stored upto 30 days at 10 °C, considering a minimum of 60% emergence percentage, which are in conformity with the present finding. Jalali and Singh (1992) [4] suggested that *T. japonicum* can be best stored effectively at 10 °C for 21 days. Nadeem *et al*. (2010) [7] showed that the maximum adult emergence (96.6%) of *T. chilonis* was recorded at 10 °C after 5 days storage, which is in agreement with the present finding at 10 °C storage for 5 days. They also revealed that, the parasitoids developed completely due to moderate temperature at 16 °C and 12 °C and emerged after 25 days and 40 days, respectively during storage, which are similar to the present findings. The data shown in Table-2 revealed that the maximum parasitization was recorded from the adults that emerged from pupae stored at 7.5 °C for 5 days (91.56%) followed by 10 °C for 5 days (90.56%) and 5 °C for 5 days (89.82%) which were at par. These parasitization data were followed by pupae stored for 10 days at 7.5 °C (87.19%), 10 °C (86.48%) and 5 °C (85.53%). There was no significant difference among these treatments. There was gradual decline in the parasitizing percentage for every temperature with the advance in storage period. The parasitization was declined to 71.74% after 30 days and 54.58% after 45 days when stored at 7.5 °C. There was also decline in parasitization at 10 °C (70.22% after 30 days and 52.22% after 45 days) and 5 °C (68.26% after 30 days and 50.22% after 45 days). The pupae stored at 12.5 °C (71.56% after 5 days) and 15 °C (64.52% after 5 days) showed comparatively lesser per cent parasitization. There was further reduction in the per cent parasitization and reached to 10.74% after 40 days of storage at 12.5 °C and 29.93% after 20 days of storage at 15 °C. The parasitization of trichocards was recorded to be nil (0.00%) after 40 days at 12.5 °C and after 20 days at 15 °C due to emergence and death of adults during storage.

The results shown above revealed that *T. japonicum* could parasitize the host (*Corcyra cephalonica*) eggs satisfactorily when pupae were stored up to 30 days at 7.5 °C followed by 10 °C and 5 °C. Khosa and Brar (2000) [5] suggested that the parasitoid could be stored at low temperature (8-10 °C) for 22 days in refrigerator without affecting its parasitization efficiency. Their results are in conformity with the present findings. The findings of Pitcher *et al.* (2002) [8] are in accordance with the present investigations on parasitisation by *T. japonicum*. They showed that there was no adverse effect on parasitization when *T. ostriniae* pupae were stored at 9 °C for 30 days whereas the parasitization per cent decreased when stored at 12 °C for 30 days. Vigneswaran *et al*. (2017) [9] reported that the highest per cent parasitism (96.00%) was observed at 10 °C for 5 days of storage which further decreased to 53.66% for 30 days of storage. These findings also support the results of the present experiments.

Table 2: Effect of storage of *T. japonicum* pupae on parasitization (%)

Duration	Parasitization (%) (in different storage temperature)					
	5 °C	**7.5 °C**	**10 °C**	**12.5 °C**	**15 °C**	**Mean**
5Days	89.82 (71.55)	91.56 (73.16)	90.56 (72.14)	71.56 (57.76)	64.52 (53.43)	81.60
10Days	85.53 (67.65)	87.19 (69.02)	86.48 (68.44)	60.63 (51.12)	56.52 (48.74)	75.27
15Days	82.82 (65.51)	84.93 (67.15)	83.63 (66.13)	48.66 (44.22)	41.37 (40.01)	68.28
20Days	78.82 (62.59)	80.63 (63.88)	79.63 (63.17)	36.60 (37.20)	29.93 (33.15)	61.12
25Days	73.71 (59.14)	75.48 (60.31)	74.44 (59.62)	29.45 (32.84)	0.00 (0.00)	50.62
30Days	68.26 (55.70)	71.74 (57.87)	70.22 (56.92)	21.93 (27.89)	0.00 (0.00)	46.43
35Days	60.56 (51.08)	66.75 (54.77)	63.60 (52.87)	17.15 (24.41)	0.00 (0.00)	41.61
40Days	55.04 (47.88)	60.26 (50.90)	57.60 (49.35)	10.74 (19.08)	0.00 (0.00)	36.73
45Days	50.22 (45.11)	54.58 (47.61)	52.22 (46.26)	0.00 (0.00)	0.00 (0.00)	31.41
Mean	71.64	74.79	73.15	32.97	21.37	
	SE(m)±			CD(0.05)		
Temp.	0.296			0.834		
Duration	0.398			1.119		
T X D	0.889			2.503		

*Figures in the parentheses are angular transformed values

Conclusion

It may be concluded that *T. japonicum* pupae can be stored up to 30 days at 7.5 °C followed by 10 °C and 5 °C without much hampering its parasitizing efficiency *viz*. adult emergence percentage and parasitizing percentage. The storage of *T. japonicum* pupae at 12.5 °C and 15 °C were proved unsuitable.

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