E-ISSN: 2618-0618
P-ISSN: 2618-060X

NAAS Rating (2025): 5.20
www.agronomyjournals.com
2025; 8(10): 98-101
Received: 14-07-2025
Accepted: 21-08-2025

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Effect of varying temperatures on *in vitro* parasitization efficiency and adult emergence of *Trichogramma japonicum* (Ashmed)

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DOI: https://www.doi.org/10.33545/2618060X.2025.v8.i10b.3959

Abstract
The present experiment was conducted in the Biocontrol laboratory, SKS CARS, Surgi, Rajnandgaon (C.G.) during the years 2023-24 and 2024-25 on effect of varying temperatures (0 °C, 4 °C, and room temperature) on the *in-vitro* parasitizing efficiency and adult emergence of *Trichogramma japonicum.* According to the findings, the highest parasitization and emergence rates were recorded in the 1st week across all temperatures, followed by a significant decline. At 0 °C, activity of parasitoids ceased after the 2nd week, indicating poor viability at this temperature. Room temperature supported only short-term viability, with complete loss of parasitization and emergence after the 1st week. In contrast, 4 °C maintained moderate levels of parasitization and emergence over the 10-week period, though both declined gradually with time. These results suggest that 4 °C is the most suitable temperature for extended storage of *T. japonicum.*

Keywords: *Trichogramma japonicum*, parasitization efficiency, adult emergence, temperature effect

Introduction
Parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are among the most extensively studied and widely utilized biological control agents against various lepidopteran pests affecting crops such as sugarcane, paddy, cotton, corn, and vegetables (Greenberg *et al*., 1996; Jalali and Singh, 1993) [7, 9]. Their popularity stems from their ease of mass rearing in insectaries and their highly efficient egg-parasitizing behavior (Li, 1994) [13]. The development of mass production and release techniques of parasitoids have promoted the use of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) species (Jalali and Singh 1992, Jalali *et al*., 2007, Firake and Khan 2014) [8, 10, 5]. Many studies have focused on their parasitizing potential, host-searching ability, and pest suppression capacity. However, the production of high-quality parasitoids for effective field release requires a better understanding of their fitness under varying environmental conditions (Nadeem *et al*., 2004) [15]. For better adaptations of natural enemies in field and *in vitro* studies are imperative. Ecological compatibility of a biological control agent is an important aspect for successful agricultural pest control. The effectiveness of several bio-control attempts has been reduced due to adverse climatic conditions. Temperature is a key factor affecting the abundance, distribution, and parasitic capabilities of egg parasitoids (Bourchier and Smith 1996; Mac eda *et al*., 2003) [].It has significant effects on developmental period and adult emergence of *Trichogramma* wasps (Foerster and Foerster 2009) [6]. In this context, the present study aimed to evaluate the effect of different temperature for storage of parasitized eggs over different durations on key fitness parameters specifically parasitization potential and adult emergence in *T. japonicum*.

Materials and Methods
Experiments were conducted to determine the optimal storage temperature and duration for *Trichogramma japonicum* during their pupal stage. Parasitized trichocards were stored under three temperature regimes *viz.*, 0 °C, 4 °C, and ambient room temperature (28 ± 2°C). To prepare

the cards, 200 eggs of *Corcyra cephalonica* were affixed to paper strips measuring 28 cm × 12 cm and exposed to one-day-old parasitoid adults of *T.japonicum* (emerged from previously parasitized cards) for 72 hours to allow parasitization. Following parasitization, the cards were subjected to the three storage temperature treatments. Each parasitized card (200 eggs) were subsequently divided into 10 equal sections, each containing 20 parasitized eggs. Beginning from the first week of storage**,** one section from each treatment group (0 °C, 4 °C, and room temperature) was removed weekly and placed into a clean zip-lock plastic bag along with a sentinel card containing 100 fresh *Corcyra* eggs. After 72 hours of exposure, parasitism was confirmed by the blackening of the eggs on the sentinel cards. These parasitized sentinel cards were then placed separately for recording the percentage of parasitization and adult emergence. This process was repeated weekly for up to 10 weeks to evaluate the effect of storage temperature and duration on parasitoid performance.

Results and Discussion

1. Parasitization of *T. japonicum* under different temperature

- **Parasitization percentage at 0 °C**

Per cent parasitization were recorded only on the 1st and 2nd week and this could not be continued from 3rd week up to 10th week as trichocards stored at 0 degree Celcius did not emerge. At 0 °C the highest parasitization percentage was recorded in 1st week *i.e.*, 2.67% which was significantly superior over 2nd week, because from 3rd to 10th week parasitization values dropped to zero (0.00). Thus, least parasitization percentage was recorded on 2nd week *i.e.*, 1.00%.

- **Parasitization percentage at 4 °C**

In 4 °C, highest per cent parasitization was recorded in the 1st week *i.e.*, 22.67% which was significantly superior with all other weeks. The weeks which showed least parasitization percentage was 10th week *i.e.*, 0.66% and was significantly lower than 1st week. The data in Table 1, shows a decreasing trend over the period of 10 weeks. The percentage parasitization was 22.67 in the first week and dropped to 0.66 by 10th week and there was significant variability in the data.

- **Parasitization percentage at room temperature (28±2°C)**

The parasitization percentage recorded at room temperature as presented in Table 1, over a period of 10 weeks indicates a significant change in conditions from the first week to the subsequent weeks. In the first week, the recorded per cent parasitization was 27.00%, which was notably higher than the consistent 0.00% parasitization recorded for each of the following nine weeks. The parasitiation remained nil from 2nd week up to 10th week.

Similar results were reported by Balabantaray and Mandal (2021) [2] stating that there was gradual decline in the parasitizing percentage for every temperature with the advance in storage period. The parasitization 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 present result contradicts with Khosa and Brar (2000) [12] who suggested that the parasitoid could be stored at low temperature (8-10 °C) for 22 days in refrigerator without affecting its parasitization efficiency.

The findings of Pitcher *et al.* (2002) [16] 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) [17] 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.

Janghel *et al.* (2019) [11] found that highest per cent parasitisation at 25 °C was observed between (F1) 82.12% to 69.25% (F10), at 28 °C the per cent parasitisation was observed between 75.03 to 60.75% (F1 to F10), at 30 °C the parasitisation was observed between 63.51 to 51.50% (F1 to F10) and at 32 °C the parasitisation was observed between 42.75% to 53.21 (F1 to F10).

Table 1: Effect of storage of *T. japonicum* pupae on parasitization (%) at different duration of time

Storage duration (In weeks)	Parasitization (%) (in different storage temperature)		
	0 °C	4 °C	Room temp. (28± 2°C)
1 weeks	2.67	22.67	27.00
	(7.62)	(28.4)	(31.27)
2 weeks	1. 00	12.00	0.00
	(4.62)	(20.16)	0.00)
3 weeks	0.00	7.33	0.00
	(0.00)	(15.46)	(0.00)
4 weeks	0.00	9.33	0.00
	(0.00)	(17.05)	(0.00)
5 weeks	0.00	8.00	0.00
	(0.00)	(16.23)	(0.00)
6 weeks	0.00	7.33	0.00
	(0.00)	(15.48)	(0.00)
7 weeks	0.00	6.33	0.00
	(0.00)	(14.29)	(0.00)
8 weeks	0.00	4.66	0.00
	(0.00)	(12.35)	(0.00)
9 weeks	0.00	1.66	0.00
	(0.00)	(6.03)	(0.00)
10 weeks	0.00	0.66	0.00
	(0.00)	(2.7)	(0.00)
CD at 5%	4.314	6.737	0.92
SE(m)±	1.452	2.268	0.31

(Figures in parentheses arc sin transformed values)

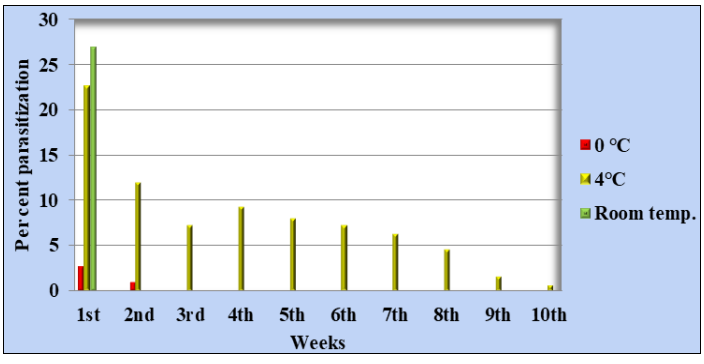

Fig 1: Effect of storage of *T. japonicum* pupae on parasitization (%) at different duration of time

2. Per cent emergence of *T. japonicum* under different temperature

- **Adult emergence percentage at 0 °C**

In Table 2, data revealed that adult emergence were observed only after one and two weeks of storage and no adult emergence was noticed from the 3rd week up to the 10th week as trichocards stored at 0 °C did not emerge. At 0 °C the maximum per cent

adult emergence was recorded in one week after storage *i.e.*, 158.00% which was significantly superior over 2nd week *i.e.*,83.00% and from 3rd to 10 th week emergence values declined to zero (0.00). Hence, least emergence percentage was recorded after two weeks of storage *i.e.*,83.00%.

- **Adult emergence percentage at 4 ℃**

At 4 °C the maximum adult emergence percentage was recorded in 1st week after storage *i.e.,* 287% which was significantly superior to all other weeks except 2nd,3rd, 4th, 5th, 6th, which was at par and least adult emergence percentage was recorded on 10 weeks after storage *i.e.*,83%.

The data in Table 2, showed a decreasing trend over the 10 weeks period for values. The values started at 287.00% in the first week and dropped to 83.00% by the 10th week. However, there was significant variability in the data.

- **Adult emergence percentage at room temperature (28±2 °C)**

The adult emergence percentage as presented in Table 2, over a period of 10 weeks indicates a significant change in conditions from the first week to the subsequent weeks. In the first week, the recorded percent emergence was 210%, which was notably higher than the consistent 0.00% emergence recorded for each of the following nine weeks. No adult emergence was recorded from the 2nd week onwards to 10th week.

According to Balabantaray and Mandal (2021) [2] maximum per cent adult emergence of *T. japonicum* 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%) revealed a gradual reduction in the emergence percentage for each temperature with increase in storage period, which is in agreement with the present finding.

Bhargavi and Naik (2015) [3] worked on the adult emergence of *T. japonicum* at 15^0C for 45 days of storage period maximum per cent were recorded at 5 days of storage (95.09%) followed by 10 days (77.72%) and were at par. Further, drastic reduction in adult emergence were noticed at 40 and 45 days of storage with 3.08 and 0.89 per cent, respectively and were at par. This is in agreement with our finding.

Abbes *et al.* (2020) [1] recorded highest mean emergence rate (88.3%) when *T. bourarachae* pupae were cold stored directly at 4 ± 1°C. Lower mean emergence rate (87.0%) was observed when pupae were subjected to pre storage period at 15 °C, while the lowest mean emergence rate (84.7%) was obtained when wasps were subjected to pre storage period at 10 °C. Statistically similar emergence rates were observed after 0 and 15days of conservation (93.8 and 92.6%) at 4 ± 1 °C respectively. Lower emergence rates of 87.56, 82.36, and 77.00% were observed after 30, 45 and 60days, respectively, of conservation at 4 ± 1 °C which is in line with the present findings.

Janghel *et al.* (2019) [11] found reduction in emergence percentage of *T. japonicum* with increase in temperature. At 25 °C was observed between 83.75 to 74.52% (F1 to F10), at 28 °C the emergence was seen between 75.61 to 68.41% (F1 to F10), at 30 °C the emergence was seen as 71.75 to 62.57% (F1 to F10) and at 32 °C the emergence was seen as 71.55 to 57.12% (F1 to F10) which is in match with the present results.

Table 2: Effect of storage duration of *T. japonicum* pupae on percentage adult emergence (%)

Storage duration (in weeks)	Adult emergence (%) (in different storage temperature)		
	0 °C	4 °C	Room temp. (28± 2 °C)
1 weeks	158.00*	287.00*	210*
	1.58	2.87	2.10
	(5.89)	(8.78)	(8.3)
2 weeks	83.00*	219.00*	0.00*
	0.83	2.19	0.00
	(4.06)	(8.49)	(0.00)
3 weeks	0.00*	158.00*	0.00*
	0.00	1.58	0.00
	(0.00)	(7.20)	(0.00)
4 weeks	0.00*	158*	0.00*
	0.00	1.58	0.00
	(0.00)	(7.10)	(0.00)
5 weeks	0.00*	148*	0.00*
	0.00	1.48	0.00
	(0.00)	(6.84)	(0.00)
6 weeks	0.00*	142*	0.00*
	0.00	1.42	0.00
	(0.00)	(6.81)	(0.00)
7 weeks	0.00*	121*	0.00*
	0.00	1.21	0.00
	(0.00)	(6.25)	(0.00)
8 weeks	0.00*	120*	0.00*
	0.00	1.20	0.00
	(0.00)	(6.21)	(0.00)
9 weeks	0.00*	120*	0.00*
	0.00	1.20	0.00
	(0.00)	(6.15)	(0.00)
10 weeks	0.00*	83*	0.00*
	0.00	0.83	0.00
	(0.00)	(5.17)	(0.00)
CD at 5%	3.53	2.36	0.45
SE(m)+	1.19	1.56	0.15

(Figures in parentheses arc sin transformed values)

(Values with * mark are the per cent emergence after multiplication with 100)

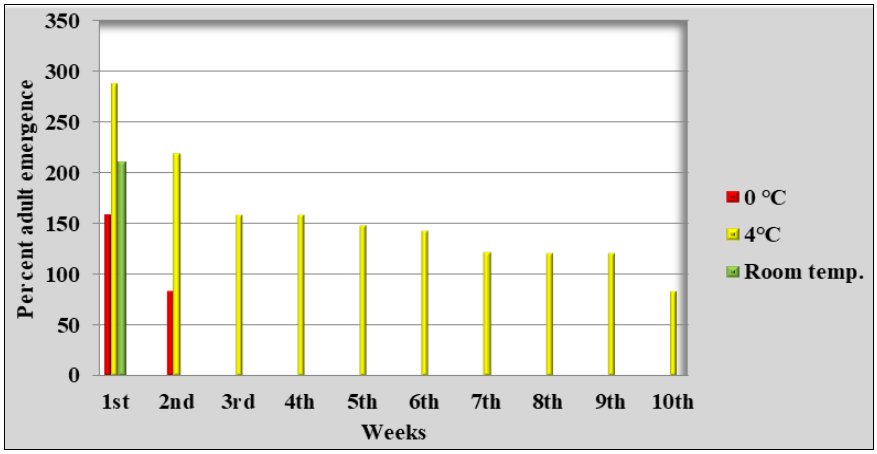

Fig 2: Effect of storage duration of *T. japonicum* pupae on adult emergence (%)

Conclusion

The present studies demonstrated that storage temperature significantly affected the parasitization and emergence potential of *Trichogramma japonicum*. Highest performance was observed during the first week across all temperature treatments, with a rapid decline in both parasitization and emergence in the subsequent weeks. At 0 °C, viability was severely reduced after the second week, while room temperature (28 ± 2 °C) supported activity only in the first week due to rapid development and depletion. In contrast, storage at 4 °C maintained moderate levels of parasitization and emergence up to 10 weeks, indicating that this temperature is optimal for short-term storage of *T. japonicum* without compromising on its biological efficiency.

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