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Biology of *Trichogramma chilonis* under varying conditions and their parasitism efficiency in lepidopteran pests

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ABSTRACT

The experiment was conducted during 2021 to 2023 at College of Agriculture (Kerala Agricultural University), Vellayani, Thiruvananthapuram, Kerala to evaluate the biology of the egg parasitoid *Trichogramma chilonis* Ishii under different conditions and its effectiveness in parasitizing lepidopteran pests. Field efficacy of *T. chilonis* in managing *Leucinodes orbonalis* Guenee, in brinjal (*Solanum melongena* L.) cv. Supriya was carried out in randomized block design (RBD) with five treatments (T_1, Trichocards @2.5 cc/ha; T_2, Trichocards @5 cc/ha; T_3, Trichocards @10 cc/ha; T_4, Chlorantraniliprole 18.5 SC 0.2 ml/litre, which was the chemical check; and Untreated control) replicated four times. Findings from the study on seasonal biology demonstrated that the developmental period was shortest in summer compared to that in monsoon and winter. Monsoon conditions were more conducive for factors such as sex-ratio, emergence rate and adult longevity, making it the optimal season for its mass production. When *Corcyra cephalonica* Stainton eggs were stored for varying durations (5, 10, 15 days) under refrigeration, it was observed that the development period of *T. chilonis* was unaffected. However, adult longevity and sex-ratio gradually decreased with an increase in storage duration, with 10 days being identified as the ideal duration. *T. chilonis* effectively parasitized eggs of various lepidopteran pests including okra shoot and fruit borer (*Earias vittella* Fabricius), brinjal shoot and fruit borer *(Leucinodes orbonalis* Guenee), tobacco cutworm (*Spodoptera litura* Fabricius), cotton bollworm (*Helicoverpa armigera* Hubner) and lemon butterfly (*Papilio polytes* Linnaeus). Field experiments indicated that controlling brinjal shoot and fruit borer infestations was successful by using trichocards at 10 cc/ha in the initial three releases and reducing it to 5 cc/ha. Plots treated with trichocards at 10 cc/ha yielded the highest quantity of marketable fruits, with all treatments proving profitable over the control. Among the different doses of trichocards, the highest dose (10 cc/ha) had the most favourable cost-benefit ratio (1:1.03). The population of natural enemies remained stable across various trichocard doses (42.25–46.25), contrasting with a notable decrease in plots treated with chlorantraniliprole 18.5 SC (23.5).

Keywords: Biology, Parasitism, *Trichogramma chilonis*

In the realm of integrated pest management (IPM) strategies, biological control is frequently regarded as a viable substitute for insecticides. The effectiveness of natural enemies in their ecological niche greatly impacts the extent of damage caused by pests in agricultural systems. Natural enemies play a crucial role in decreasing the prevalence of insect pests, despite the widespread application of broad-spectrum insecticides. Among the different natural enemies employed for pest management, the genus *Trichogramma* known as the living insecticide, is one of the promising ones (Shankar 2017). A multitude of trichogrammatid species have been identified globally, playing a pivotal role in the biological management of lepidopteran pests across various crops through laboratory-scale breeding and subsequent augmentative releases (Nagaraja 2013).

T. chilonis is prevalent throughout the country and its large-scale field introduction have shown significant success in decreasing the population density of many lepidopteran pests. Unlike many other species, it has a broad range of host insects and is recognized for its ability to target over 200 lepidopteran species, such as loopers, borers, cutworms, bollworms, armyworms, and others (Ahmed *et al*. 2023). In India, approximately 45 species of *Trichogramma* are found in their natural habitat, while globally, there are around 240 described species of *Trichogramma* (Khan *et al*. 2020). Validation of efficacy of this parasitoid, which is amenable to mass production, gains importance in the context of production of safe to eat vegetables. Uninterrupted supply of these biocontrol agents for scheduled inundative releases needs refinement of its production strategies. The present study therefore identified the ideal season for mass

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multiplication *T. chilonis*, storage condition of its host eggs, parasitism efficiency in various lepidopteran pests of vegetables and field efficacy in managing *L. orbonalis* infestation in brinjal. The findings derived from this investigation would serve as an encouraging measure in the direction of ecologically conscious pest management for both residential areas and agricultural settings that cultivate vegetables for homestead as well as commercial purposes.

MATERIALS AND METHODS

The experiment was conducted during 2021–23 at College of Agriculture (Kerala Agricultural University), Vellayani, Thiruvananthapuram, Kerala.

Maintenance of insect culture: The culture of *T. chilonis* and the factitious host, *C. cephalonica* maintained in the AICRP on Biological Control of Crop Pests, Kerala Agricultural University, Vellayani [originally sourced from ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru] was used for the study. A controlled environment with a temperature of 28 ± 2°C and a relative humidity (RH) of 75 ± 5% was maintained throughout the study, by using temperature and humidity control devices such as air conditioner and humidifier, respectively for rearing the host, *C. cephalonica*.

Biology: The host eggs were pasted in mini paper cards which were subjected to parasitization by releasing two pairs of newly emerged *T. chilonis,* in a parasitisation chamber. The observations on the egg, larval and pupal periods, the total developmental period, emergence rate, adult longevity and sex ratio were recorded. After attaining the pupal stage, as indicated by the blackening of the host egg chorion, the egg cards were cut into small bits carrying a single egg. They were then placed in small glass vials to observe the adult emergence. The adults were sexed based on their morphological characters as described by Nagarkatti and Nagaraja (1977) by observing under a stereo-zoom microscope (Carl Zeiss).

Biology of *T. chilonis* was studied during three different seasons, summer (March–May), winter (December–February) and monsoon (June–August). The average temperature during summer was 32.50°C and relative humidity was 75.70%. During monsoon, it was 29.50°C and 84.80% and during winter it was 27.10°C and 74.50%, respectively. The effect of storage period of *Corcyra* eggs on the biology of *T. chilonis* was observed by parasitizing the un stored eggs and eggs stored under refrigeration (5°C) for a period of 5, 10 and 15 days.

Parasitism efficiency: The parasitism efficiency of *T. chilonis* in different lepidopteran eggs under laboratory conditions was examined in the eggs obtained from laboratory culture of the reared in their host plants or in the eggs collected from field. The laboratory collected eggs were pasted on mini egg cards and were subjected to parasitization with two pairs of newly emerged *T. chilonis*. The field collected ones were observed for any natural, or pre parasitism before releasing *Trichogramma*. Parasitism efficiency was calculated as:

$$\text{Parasitism per cent} = \frac{\text{Number of eggs parasitized} \times 100}{\text{Total number of eggs kept for parasitization}}$$

$$\text{Adult emergence (\%)} = \frac{\text{Number of parasitoids emerged} \times 100}{\text{Number of eggs parasitized}}$$

The total duration (days) between parasitization and adult emergence was counted as the period of emergence. Sex differentiation of emerging adults was done primarily based on their morphology.

Field efficacy: Field efficacy of *T. chilonis* in managing *L. orbonalis* in brinjal was carried out in randomised block design (RBD) with five treatments, each replicated four times. Each replication carried six plants. A distance of 1.20 m was kept in between the treatment plots. The Kerala Agricultural University variety Supriya (Brinjal variety) was used for the study. The treatments were, T_1, Trichocards @2.5 cc/ha; T_2, Trichocards @5 cc/ha; T_3, Trichocards @10 cc/ha; T_4, Chlorantraniliprole 18.5 SC 0.2 ml/litre, which was the chemical check; and Untreated control.

Six releases of *T. chilonis* were done each at an interval of one week. The number of parasitised eggs released was according to the treatment doses and plot size. The doses were, 2.5 cc/ha (50,000 parasitised eggs); 5 cc/ha (1 lakh parasitised eggs); and 10 cc/ha (2 lakh parasitised eggs). After each release, the plots were covered using nylon nets to prevent the movement of *T. chilonis* adults to the adjacent plots and the nets were removed after four days of release until the next release. Observations on infested fruits count were taken seven days after each release, at the time of fruit picking.

$$\text{Fruit infestation per cent} = \frac{\text{Number of damaged fruits} \times 100}{\text{Total number of fruits}}$$

The mean marketable yield/plot was calculated at weekly intervals. The cost-benefit ratio of brinjal cultivation by employing trichocards was computed by the method followed by Singh *et al.* (2019). The natural enemy population from each plot was estimated by visual observation of the total number of coccinellids and predatory spiders.

Statistical analysis: The data obtained from the experiment were subjected to analysis of variance (ANOVA), using WASP1 software.

RESULTS AND DISCUSSION

Biology: The study on biology carried out during the three different seasons revealed that the seasonal temperature has a significant role in the developmental period, sex-ratio and adult longevity of *T. chilonis.* The emergence rate exhibited a decreasing trend with an increase in temperature. The developmental period was shortest in the summer. Monsoon conditions were more favourable for factors such as sex ratio, emergence rate, and adult longevity, making it the optimal season for the mass reproduction of *T. chilonis* (Table 1).

Table 1 Biology of *Trichogramma chilonis* under varying conditions

Observations	During different seasons					During different storage conditions of *Corcyra* eggs					
	Summer	Monsoon	Winter	SE(m)	CD	0 days	5 days	10 days	15 days	SE (m)	CD
Emergence rate (%)	80.39 (8.96)	88.92 (9.42)	87.47 (9.35)	3.234	NS	88.09 (9.37)	87.54 (9.35)	85.12 (9.22)	81.75 (9.04)	2.743	NS
Development period (days)	6.08[c]	7.13[b]	7.92[a]	0.052	0.16	7.25	7.21	7.27	7.28	0.064	NS
Adult longevity (days)	2.47[b]	3.43[a]	3.55[a]	0.044	0.13	3.48[a]	3.48[a]	3.46[b]	3.36[c]	0.031	0.09
Sex ratio male: female	1: 1.84	1: 2.23	1: 2.02	-	-	1:2.20	1:2.00	1:1.80	1:1.55	-	-

Mean of five replications, each replication with 20 eggs, Figures in parenthesis are values after square root transformation.

Such alterations in the developmental duration, adult longevity and sex ratio of *T. chilonis* was reported in the studies carried out by Miura and Kobayashi (1993) in the eggs of diamond back moth, *Plutella xylostella* L. They noted that the developmental period was drastically shortened from 18.7–6.5 days when the temperature was increased from 17–32°C. An acceleration in the metabolic activity of the juvenile phases at elevated temperatures could be the reason behind the reduction in the duration of the developmental period as suggested by Bueno *et al*. (2009).

Nadeem and Hamed (2008) noted that with the increase in temperature from 15– 35°C, adult longevity of *T. chilonis* decreased from 5.6–1.6 days. The potential cause for the rise in adult longevity at lower temperatures was be attributed to the decrease in parasitoid activity and metabolic rate during periods of low temperature as proposed by Sajid *et al*. (2009). In a comparative study conducted by Alloui-Griza *et al.* (2022), it was revealed that the highest emergence of *T. cacoeciae* was recorded at a temperature of 25°C (80–90%) and the lowest emergence was recorded at a higher temperature of 35°C (55–60%). At 40°C, there was no emergence of the parasitoid. The impact of temperature in sex allocation of the parasitoid was established in the experiment conducted by Augustin *et al*. (2022). They observed that increased temperature results in decreased mating success and sperm transfer in haplodiploids, which ultimately results in an increased male population.

Eggs of *C. cephalonica* are extensively utilized for large-scale production of *T. chilonis*. For the continuous production of *T. chilonis*, cold storage of *Corcyra* eggs is imperative, to facilitate uninterrupted provision of the host eggs. The biology of *T. chilonis* revealed that the developmental period was unaffected by storage up to 15 days. Though there was no significant variation in emergence rate there was a decreasing trend with an increase in storage period. The adult longevity and sex ratio were negatively affected by the extended storage (Table 1). In the study 10 days of storage was identified as the ideal storage duration without much impact on the emergence, sex-ratio and adult longevity.

These observations align with the study of Wu *et al*. (2018) who noted that the developmental period and emergence rate of *T. chilonis* did not exhibit any noteworthy impact due to the cold storage of *C. cephalonica* eggs up to 15 days. Siam *et al.* (2019) noted a slight decrease in the emergence rate of *T. evenescence* from 96.92–94.85% after a period of five days of storage. When the storage period was further increased to 10, 15 and 30 days, a corresponding decrease in the emergence rate (84.91%, 80.48% and 50.73%, respectively) was noted, substantiating that changes in host egg quality by prolonged storage affect the emergence rate of the parasitoid. The findings of Shawer *et al*. (2022) unveiled that the longevity of *T. evanescence* adults, regardless of the duration of storage of host eggs, exhibited a significantly lower value compared to that of non-refrigerated eggs.

Parasitism efficiency: The parasitism efficiency of *T. chilonis* in eggs collected from laboratory culture of *E.vittella*, *L. orbonalis*, *H. armigera* and *S. litura* (Table 2), indicates that *T. chilonis* exhibited a differential range of parasitism in different host insects, ranging from 90% in *E. vittella* to 60% in *H. armigera*. However, it did not parasitize the eggs of cucumber moth, *Diaphania indica* Saunders and okra leaf roller, *Sylepta derogata* F. Among field-collected lepidopteran eggs (Table 2), lemon butterfly *Papilio polytes* L. exhibited 88.89% parasitism, while in castor hairy caterpillar *Pericallia ricini* F. was 63.39% and in spiny caterpillar *Ergolis merione* Cramer it was very less as 15.0%.

Review of existing literature also pointed out that, the parasitism rates of *T. chilonis* varied according to the host insect. The selective preference of parasitoids towards certain eggs over others and subsequent differential parasitism may be due to the chorion architecture, thickness, size and shape of host eggs as suggested by Schmidt (1994). Mehendale (2009) observed 94.98% parasitism by *T.chilonis* in *H. armigera*, 91.97% in *E. vittella* and 72.98% in *S. litura*. Honnayya and Gawande (2018) noted 78.75% parasitism in *E. vittella,* 86.25% in *H. armigera* and 81.25% in *Papilio* sp. Funde *et al*. (2020) reported 46.31% parasitism in *E. vittella*, 62.39% in *H. armigera* and only 10.35% in *L. orbonalis*. They opined that the emergence rate was not profoundly affected by the host egg and its age.

The period of emergence of *T. chilonis* from different lepidopteran eggs as noted by Honnayya and Gawande (2018) as 7.00 days in *H. armigera*, 7.50 days in *E. vittella*

Table 2 Parasitism efficiency of *T. chilonis* in lepidopteran eggs

Host insects	Parasitism (%)*	Emergence (%)**	Period of emergence (days)	Sex ratio (male: female)
Parasitism (%) in laboratory reared host insects				
E. vittella	90.00 (73.45)[a]	91.33 (9.55)	8.39 ± 0.09 [a]	1: 1.92
H. armigera	60.00 (50.82)[c]	86.95 (9.31)	8.34 ± 0.12 [a]	1: 1.89
L. orbonalis	84.00(66.69)[ab]	90.56(9.51)	8.37 ±0.10 [a]	1: 1.85
S. litura	78.00(62.40)[b]	95.00(9.74)	7.54 ± 0.04 [b]	1: 1.47
C. cephalonica	87.5(71.37) [ab]	89.36(9.43)	7.13 ± 0.10 [c]	1: 2.20
D. indica	0.00(0.91)[d]	-	-	-
S. litura	0.00(0.91)[d]	-	-	-
SE (m)	3.279	2.783	0.043	-
CD (*P*=0.05)	(8.111)	NS	0.127	-

Parasitism (%) in field-collected eggs

Host plant	Host insect	Nature of egg	Parasitism (%)
Castor	*P. ricini*	Egg mass	63.39
Curry leaves	*P. polytes*	Single,scattered	88.89
Castor	*E. merione*	Single,scattered	15.00

Mean of five replications each replication contains 10 eggs; *, Figures in parentheses are values after Arcsin transformation; **, Figures in parentheses are values after square root transformation.

and 7.50 days in *C. cephalonica*. Funde *et al*. (2020) noted 8.33 days in *H. armigera*, 7.66 days in *L. orbonalis*, 8.33 days in *E. vittella* and 8.66 days in *C. cephalonica* which are in agreement with the present findings.

Field efficacy: When the percentage of fruits infested were analyzed (Table 3), it was observed that among different doses of trichocards, the highest dose of 10 cc/ha resulted in the lowest fruit infestation up to the first three releases. From the fourth release onwards it was in parity with that of the next lower dose (5 cc/ha). After the sixth release, these two treatments became on par with each other and were in parity with the chemical check, Chlorantraniliprole 18.5 SC 0.2 ml/litre plots. This indicated that a dose of 10 cc/ha during the initial three releases and next lower dose of 5 cc/ha during the subsequent release can effectively manage the fruit infestation in brinjal. The percentage reduction in fruit infestation over control (Fig. 1) revealed that, among different doses of trichocards, the highest reduction in infestation was in plots treated with 10 cc/ha (46.36%) followed by that treated with 5 cc/ha (34.41%). The lower dose of 2.5 cc/ha was found to be less effective (20.54%). Percentage reduction in infestation noted in chlorantraniliprole 18.5 SC (0.2 ml/litre) treated plots was 58.83%.

Biswas *et al.* (2021) evaluated different doses of *T. chilonis* for the management of fruit and shoot borer in brinjal and noted that the infestation decreased in plots treated with all doses of *T. chilonis*. The highest reduction over control was observed in plots with a release rate of *T. chilonis* 5 cc/ha (77.68%), followed by that in 3.75 cc/ha (75.26%). Least reduction over control was observed in the plots with the lowest release rate of 2.5 cc/ha (69.24%).

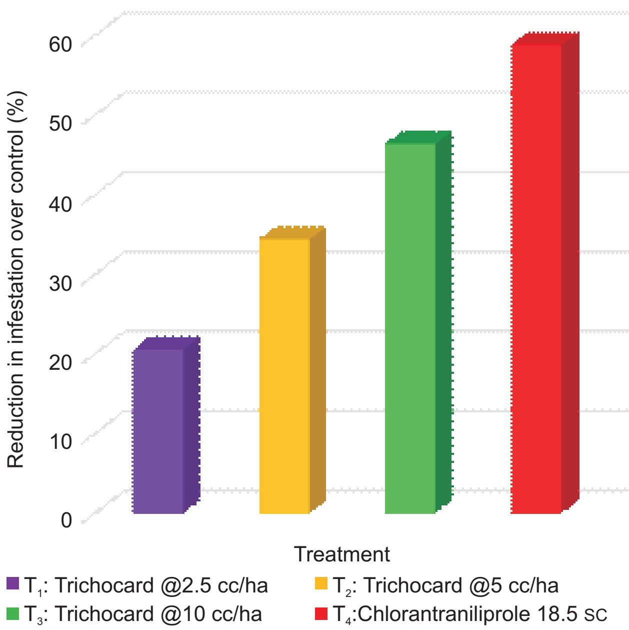

Fig. 1 Effect of *Trichogramma chilonis* on percentage reduction in fruit infestation over control.

Similarly, the yield data revealed that the average marketable yield of brinjal (Fig. 2) in plots treated with 10 cc/ha was higher than those obtained from plots treated with 5 cc/ha and 2.5 cc/ha. During the sixth and seventh harvests, the yield from plots treated with trichocards at 10 cc/ha was in parity with the insecticide-treated plots. The cost-benefit ratio (Table 3) of brinjal cultivation involving different doses of trichocards revealed that all the treatments were profitable compared to untreated control plots. Among

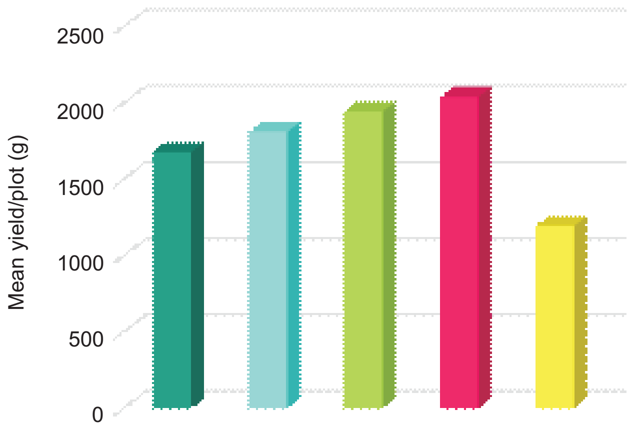

Fig. 2 Effect of *Trichogramma chilonis* on mean yield of brinjal.

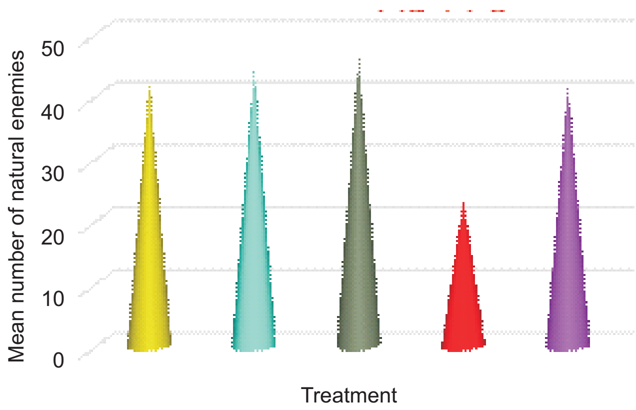

Fig. 3 Effect of *Trichogramma chilonis* and chemical treatment on the population of natural enemies.

Table 3 Effect of *T. chilonis* egg cards on *Leucinodes orbonalis* infestation in brinjal

Treatment	Infestation in fruits (%) at weekly intervals							C:B ratio
	Pre-release	Post-release						
		1	2	3	4	5	6	
Trichocards @2.5 cc/ha	35.66	35.16	33.18^{a}	31.04^{ab}	27.08^{ab}	24.64^{ab}	22.50^{ab}	1:1.02
Trichocards @5 cc/ha	36.64	34.67	31.10^{ab}	23.95^{bc}	22.22^{bc}	18.60^{bc}	12.74^{b}	1:1.02
Trichocards @10 cc/ha	33.63	30.64	23.61^{bc}	21.81^{cd}	17.95^{bc}	13.99^{bc}	9.17^{b}	1:1.03
Chlorantraniliprole 18.5 SC 0.2 ml/litre	34.29	25.35	20.97^{c}	16.25^{d}	13.99^{c}	7.14^{c}	6.25^{b}	1:1.06
Untreated control	35.92	35.07	33.18^{a}	36.76^{a}	37.35^{a}	39.02^{a}	37.08^{a}	-
SE (m)	2.181	4.765	2.442	2.446	3.372	4.887	5.298	-
CD (P=0.05)	NS	NS	7.526	7.535	10.39	15.059	16.325	-

Mean of four replications; six plants/replication.

different doses of trichocards, the highest cost-benefit ratio (1:1.03), was obtained with a dose of 10 cc/ha. In plots released with 5 and 2.5 cc/ha it was 1:1.02. The data on the natural enemy predatory spiders, viz. *Oxyopes shweta*, *O. birmanicus*, *Neoscona mukherjee*, *Plexippus petersi*, *Thanatus elongatus*, *Uloborous* spp. and *Hyllus* spp. and predatory coccinellid (*Cheilomenes sexmaculata*) count in *Trichogramma* released and insecticide treated plots revealed that the placement of trichocards did not result in any detrimental effects on their population. Nevertheless, the average population noted remained consistent across the various doses of trichocards employed, whereas the population markedly diminished in those plots administered with chlorantraniliprole 18.5 SC (Fig. 3).

In a study to evaluate the efficacy of *T. chilonis* in managing *L. orbonalis*, Reddy *et al.* (2013) recorded the highest yield of 167.38 q/ha in plots treated with the highest dose of *T. chilonis* @7.5 cc/ha, followed by the plots treated with *T. chilonis* @6 cc/ha (159.38 q/ha). Among different doses of *T. chilonis,* the lowest yield was recorded from the lowest dose of 5 cc/ha (129.92 q/ha). Singh *et al.* (2019), noted that all the doses of trichocards were economically advantageous compared to the control plots. The most substantial net profit and the most favourable cost-benefit ratio of 1:1.31 were observed when *T. chilonis* was released eight times at a rate of 7.5 cc/ha. Conversely, for the same releases, the smallest net profit and thereby the least favourable cost-benefit ratio of 1:1.025 were documented with the lowest release rate of 2.5 cc/ha. So also, for six releases the most favourable cost-benefit ratio of 1:1.030 was observed when *T. chilonis* was released at a higher rate of 7.5 cc/ha and the least favourable cost-benefit ratio of 1:1.024 was documented for *T. chilonis* at a lowest dose of 2.5 cc/ha, as similar to that observed in the current study.

The present investigation concludes that monsoon appeared to be the ideal season for the mass multiplication of *T. chilonis* compared to the other two seasons. For mass multiplication of *Trichogramma*, *Corcyra* eggs can be safely stored for up to 10 days under refrigeration without adversely affecting the sex ratio or adult longevity. *T. chilonis* can efficiently parasitize the eggs of many lepidopteran pests found in vegetable crops, viz. *E. vittella*, *L. orbonalis*, *S.*

litura, *H. armigera* and *P. polytes* and hence can be widely exploited for their management. For inundative release of *Trichogramma* in managing *L. orbonalis* infestation, cards with 10 cc/ha can be recommended during the initial three releases, while the subsequent releases may be reduced to 5 cc/ha for effective management.

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