

Biological parameters and thermal requirements of *Trichogramma chilonis* reared on *Corcyra cephalonica* and *Plutella xylostella* eggs

L Jidung, SM Haldhar, KI Singh, B Ushasri, S Saravanan & LNK Singh

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Biological parameters and thermal requirements of *Trichogramma chilonis* reared on *Corcyra cephalonica* and *Plutella xylostella* eggsL Jidung¹, SM Haldhar¹✉, KI Singh¹, B Ushasri¹, S Saravanan¹ & LNK Singh²¹Department of Entomology, College of Agriculture, Central Agricultural University, Imphal, India, 795004²Department of Pathology, College of Agriculture, Central Agricultural University, Imphal, India, 795004✉ Corresponding author: SM Haldhar, E-mail: haldhar80@gmail.com**Article Info****Article history**

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Key

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Words:**Abstract**

The objective of this work was to determine the biological parameters and thermal requirements of *Trichogramma chilonis* strain reared on *Corcyra cephalonica* and *Plutella xylostella* eggs at College of Agriculture, CAU, Imphal, Manipur. The optimum temperature for the parasitism and emergence of *T. chilonis* was 25°C and the utmost longevity was found at the same temperature. Maximum parasitism was observed 84.14 and 82.71 per cent at 25°C, while minimum parasitism was 76.57 and 69.14 per cent at 20°C on *C. cephalonica* and *P. xylostella* eggs, respectively. The results indicate that temperature has a significant effect on the biological parameters of *T. chilonis* and with increasing temperature developmental duration decreased in laboratory condition of Manipur. On the basis of the present results *Corcyra cephalonica* eggs was more suitable for mass production of *T. chilonis* than *P. xylostella* under controlled conditions.

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Introduction

Insect-pests carry on influencing agricultural production and human health significantly from many decades (Sharma 2013). Pesticide control of pests is a ordinary practice to minimize crop losses (Shah et al. 2020; Gul et al. 2021); tranquil their impacts on the soil, environment, non-target organisms, and the existence of residues in food products are matters of worldwide concern (Kumar et al. 2012; Taning et al. 2019; Varikou et al. 2019). Moreover, insecticide induced resistance and hormesis are reflection to be significant factors in the breakdown of chemical based insect-pest

control (Ullah et al. 2021). Alternatively, biological control agents have the probable to reduce crop losses and the use of pesticides (Haldhar et al. 2020 a&b; Gowda et al. 2021). Of the different bioagents, the family Trichogrammatidae (Order: Hymenoptera) has few unbeaten biological control agents for agricultural and forest pests (Qu et al. 2020; Singh & Haldhar 2020), the largely main genus under the family being *Trichogramma* (Zang et al. 2021). Globally, *Trichogramma* spp. is a stronghold of augmentative biological control and is yearly applied to 15 million hectares across 40 countries (Zang et al.

2021). *Trichogramma* is extensively released in India to stifle different caterpillar pests attacking rice, maize, cotton, sugarcane, vegetables, and fruits. The tiny generation time and mass rearing on factitious hosts tolerate *Trichogramma* to be produced rapidly and affordably compared to other parasitoids (Wang et al. 2020).

Egg parasitoid, *Trichogramma* are known to be very successful against a number of crop pests. *Trichogramma* are extremely tiny wasps of size 0.2 to 1.5 mm belonging to the family Trichogrammatidae of the order Hymenoptera. The member of this family is recognized by the three segmented tarsi (Haldhar & Deshwal 2017). These wasps naturally occur in almost all terrestrial habitats and some aquatic habitats also. It is a gregarious endoparasitoids and polyphagous, specifically parasitizing lepidopteran eggs. It is the widely used natural enemy of pests owing to its rearing abilities in insectaries and ravenous parasitizing tendency on eggs of variety of target hosts (Haldhar et al. 2020c). Amongst the *Trichogramma*, in India mainly leading and common species is *Trichogramma chilonis* Ishii. *Trichogrammatids* are one of the most important groups of bio-agents with renowned interest for the suppression of lepidopteran pests all over India. Today, *Trichogramma* species are the most widely used insect natural enemy in the world, partly because they are easy to mass rear and these attack many important crop insect-pests. As a result it is considered as a living biological insecticide that attacks only the target pest without affecting other beneficial insects, human health or the surrounding environment (Knutson 1996). The present studies were proposed to find out most suitable host out of biological parameters and thermal requirements of *T. chilonis* strain reared on *C. cephalonica* and *P. xylostella* eggs for quality mass production of parasitoid

keeping in view high per cent parasitism and adult emergence for future need of organic production in NEH region of India.

Materials and Methods

Insects rearing

For the present research, the cultures of *P. xylostella* and *C. cephalonica* were maintained beneath laboratory condition on the natural diet. The rearing temperature was maintained at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $65 \pm 5\%$ relative humidity at Bio-control Laboratory, Department of Entomology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India during the months of November to March, 2021-2022.

Mass rearing of *Corcyra cephalonica* culture

The culture of *C. cephalonica* was maintained under the same laboratory conditions. For its maintenance, 1 cubic centimetre of *Corcyra* eggs was sprinkled per wooden box containing 2.5kg of sterilized broken maize (culture medium) and was mixed up thoroughly followed by covering the box with lid. The larvae fed on broken maize until it went for pupation. Moth started emerging within 45-50 days, the moths were collected inside the net by glass tubes and transferred the moths to egg laying chamber. The adult female laid eggs inside the egg laying chamber then eggs were collected daily with brush and used for further experimental purpose.

Mass rearing of *Trichogramma chilonis* culture

The laboratory strain of *T. chilonis* was procured from ICAR-NBAIR, Bengaluru and the successful generations were maintained at the lab by employing the eggs of *C. cephalonica* pest under the same laboratory conditions. Around 0.5cc of eggs was used for a single card (1cc of eggs consists of around 16000-18000 eggs). The card was then inserted to a glass tube where the adults of

Trichogramma are present and was allowed for parasitisation. Adults *T. chilonis* usually emerged after 8-9 days and were provided with new fresh cards for culture maintenance.

Mass rearing of *Plutella xylostella* culture

To maintain DBM eggs for experiment, larvae and pupae were collected from the cabbage fields and cabbage leaves were supplied as food for the larvae. The emerged adults were released into a rearing cage (34 cm x 34cm x 26cm) (Miura & Kobayashi 1993). The cage contained planted mustard in a small pot for the adult oviposition and source of food.

The host eggs after turning black were counted for calculating %

$$\text{Per cent parasitization} = \frac{\text{No. of parasitized eggs in trichocards}}{\text{Total no. of eggs in trichocards}} \times 100$$

Development time = Number of days required for development from egg to adult emergence from egg

$$\text{Per cent Emergence} = \frac{\text{No. of adults emerged from trichocards}}{\text{Total no. of parasitized eggs in trichocards}} \times 100$$

Adult longevity = Number of days until the death of adults

Data analysis

Transformations (angular and square root transformed values) were used to accomplish normality in the data before analysis, but untransformed means were also present in all the tables. The data obtained from recording of various activities of *T. chilonis* observed at different temperature from the laboratory studies were statistically analysed as per CRD design. The means of significant parameters among tested cultivars were compared using Tukey's honestly significant difference (HSD) tests for paired comparisons at a probability level of 5%.

Results and Discussion

The results of the biological parameters such as parasitization rate, emergence rate, developmental period and longevity of *T. chilonis* on the eggs of *C. cephalonica* and *P.*

Effect of temperatures on biology of parasitoid

The parasitisation capacity of *T. chilonis* was analyzed at different temperature using the fresh eggs of laboratory host, *C. cephalonica* and *P. xylostella*. One hundred eggs of each host was glued to a card of size (4cm X 0.5cm) and introduced into a separate glass vial (5cm x2cm), where the male and female adult parasitoids were allowed to mate earlier. The entire experimental set up was carried out at three different temperature regimes, 20°C, 25°C and 30°C using an incubator. The eggs which turned black in colour were considered as parasitized eggs.

xylostella at three different temperature are depicted in the Table 1 and 2.

Biological parameters of *T. chilonis* on *C. cephalonica* eggs at different temperature

Per cent parasitization of *T. chilonis* on *C. cephalonica* eggs

The data pertaining to biological parameters of *T. chilonis* on *C. cephalonica* eggs at three different temperatures are shown in Table 1. It was observed that out of three temperatures, the maximum per cent parasitisation was found at 25°C (84.14%) followed by 30°C (80.29%) and minimum rate of parasitism was observed at 20°C (76.57%). The rate of parasitism by *T. chilonis* was significantly different at three temperatures. The result further indicates that at high temperature, rate of parasitism by *T. chilonis* decreased significantly. These results are in

harmony with findings of (Janghel et al. 2019) who documented that maximum and significantly superior per cent parasitization of *T. chilonis* was noticed at 25°C and revealed that with higher temperature the percent parasitisation, emergence of adult, development time and longevity of *T. japonicum* and *T. chilonis* decreased. Garcia & Tavares (1994) also founded that *T. chilonis* parasitized more eggs at 26°C than at the other temperatures which is in close proximity with our present findings. However, Calvin et al. (1984) reported 30°C was the most suitable temperature for *T. chilonis*. Similarly, Perveen et al. (2012) reported that maximum parasitization of *T. chilonis* was observed on *C. cephalonica* eggs (84.3 per cent) at 28°C. Venkatesan & Jalali (2013) also observed that the effects of the two physical factors-temperature and humidity on the biology of *Trichogramma* have shown that temperature below 15 or above 35 °C tends to cause mostly negative effects on their survival and/or performance. The findings of present study made it clear that 25°C is best suited for the highest per cent parasitisation and yielded maximum adults of *T. chilonis*. However, at 20°C it was least favoured for parasitization and yielded minimum adults of *T. chilonis*.

Per cent adult emergence of *T. chilonis* on *C. cephalonica* eggs

The results indicate that maximum adult *T. chilonis* emergence at different temperature from parasitized eggs was noticed at 25°C (81.29 %) followed by (69.43 %) at 30°C and lowest adult emergence was recorded at 20°C (63.57%) which were significantly different at different temperature levels. Similarly, Miura & Kobayashi (1993) documented that percentage of adult emergence of *T. chilonis* decreased as the temperature increased from 20°C to 32°C and decreased as the temperature went from 20°C to 17°C. The present result got the support from the

findings of (Janghel et al. 2019) who noticed highest adult emergence of *T. chilonis* at 25°C (80.41 %) and lowest emergence was recorded at 32°C (63.75%) when reared on *C. cephalonica* eggs.

Adult longevity of *T. chilonis* on *C. cephalonica* eggs

The adult longevity at 20°C was observed highest i.e. (4.14 days) a followed by (3.43 day) at 25°C and the lowest was observed at 30°C i.e. (2.71 days). Similarly, Shirazi (2006) have reported that the longevity of *Trichogramma* adults was decreased as the temperature increased from 20 to 30°C which corroborates present findings. However, Nadeem et al. (2010) reported that adult longevity was about 9 days at 28°C followed by 10 days at 25°C, whereas rearing at 40°C did not support the survival of parasitoids. Harrison et al. (1985) also reported that the adult longevity was decreased with increase in temperature supporting present findings. The effect of temperature on *T. chilonis* on *C. cephalonica* is also at par with most of earlier studies. Thus, the present study indicate that the temperature of 20°C was found best for adult longevity of *T. chilonis* among all the temperatures tested (Table 1).

Developmental period of *T. chilonis* on *C. cephalonica* eggs

The time taken by the parasitoid to develop into adult from an egg stage was recorded as 8.57, 8.14 and 5.43 days at 20°C, 25°C and 30°C respectively. This finding signifies that the time taken for the complete development of the parasitoid decreased as the temperature increased. According to (Jervis 2012) there is an optimal range of temperature for insect development, beyond which they would be unable to continue oogenesis and laying eggs or unable to function appropriately for a long period of time. The developmental period of *T. chilonis* inside the host eggs at 20°C was 8.57 days and it was

evaluated as highest when compared to developmental period at other rearing temperatures. The shortest developmental period (5.43 days) was recorded at 30 °C. Harrison et al. (1985) observed that the development of *T. exiguum* was faster at 25 and 30°C while it was slow at 20°C. Sultan et

al. (2013) documented that temperature has a significant effect on the biological parameters of *Trichogramma* and with increasing temperature developmental duration decreased. Thus, the developmental time of *T. chilonis* in present findings decreased with the increase in temperature.

Table 1. Per cent parasitization, developmental period (egg to adult), emergence rate and adult longevity of *T. chilonis* on *C. cephalonica* at different temperatures

Temp(°C)	Parasitization capacity %	Developmental period (days)	Emergence rate %	Longevity period (days)
20	76.57 (61.07) ^a	8.57 ^b	63.57 (52.89) ^a	4.14 ^c
25	84.14 (66.64) ^b	8.14 ^b	81.29 (64.39) ^c	3.43 ^b
30	80.29 (63.72) ^a	5.43 ^a	69.43 (56.44) ^b	2.71 ^a
SEM (±)	0.90	0.18	0.54	0.07
SE(d)	1.27	0.26	0.76	0.10
LSD (P = 0.05)	2.67	0.55	1.59	0.20

Figures in parenthesis area are angular transformation values

Value following different letter down the column are significantly different using Turkey's HSD test

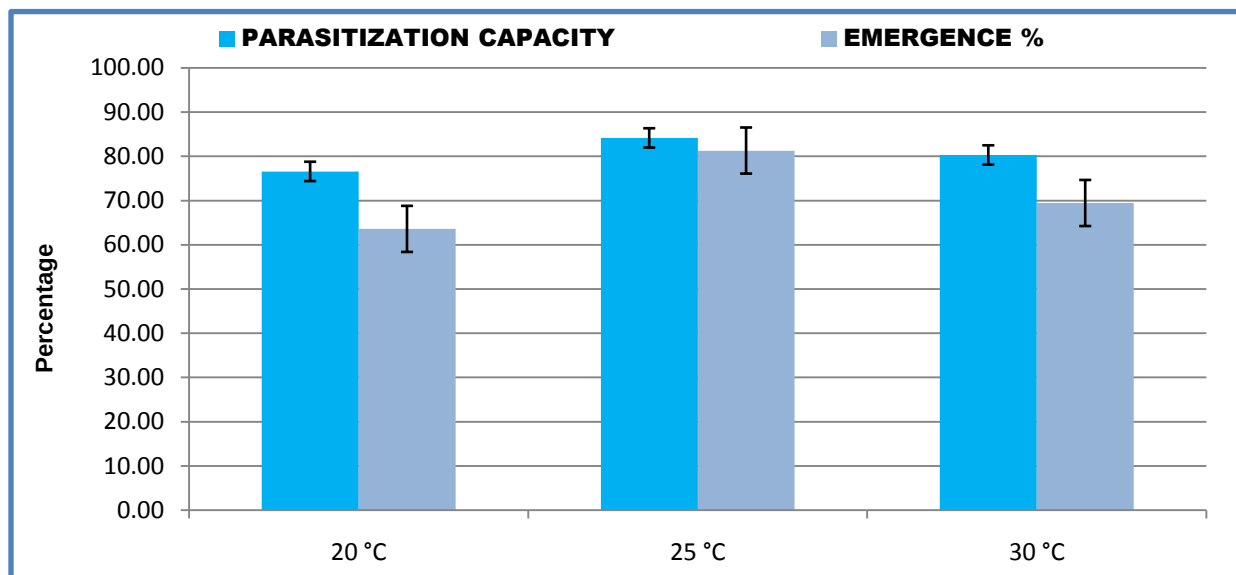


Figure 1. Graphical representation on parasitization and emergence rate of *T. chilonis* at different temperatures on *C. cephalonica* eggs

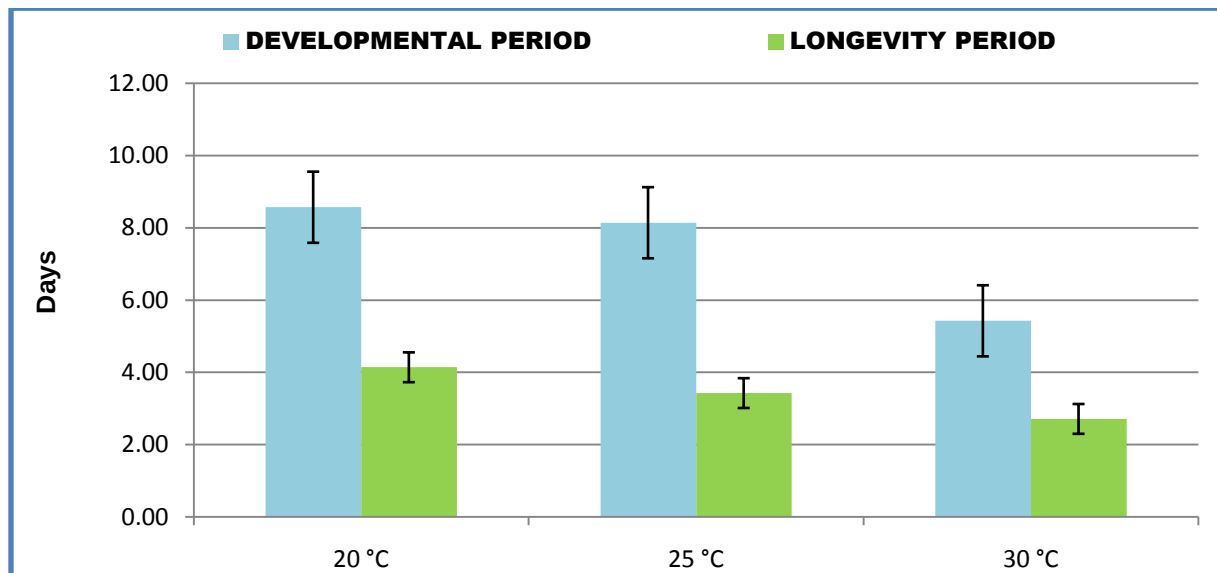


Figure 2. Graphical representation on developmental period and longevity period of *T. chilonis* at different temperatures on *C. cephalonica* eggs

Biological parameters of *Trichogramma chilonis* on *Plutella xylostella* eggs at different temperature

Per cent parasitization of *T. chilonis* on *P. xylostella* eggs

On *P. xylostella* eggs, maximum parasitism 82.71% was observed at 25°C followed by 77.57% at 30°C, while minimum parasitism was 69.14% at 20°C. The parasitism was non-significant to each other at 25 and 30°C, while, it was significantly different at 20°C. However, Miura & Kobayashi (1993) demonstrated that 28°C was the optimum temperature for *T. chilonis* developing on eggs of diamond back moth. Perveen et al. (2012) reported that most favourable temperature was 28°C on which maximum parasitism and adult emergence were obtained from *S. cerealella*. The results indicate that at high temperature, parasitism was decreased by *T. chilonis*. Similar results were found for *C. cephalonica* eggs. The results indicate that maximum parasitism was 84.14% at 25°C followed by 80.29% at 30°C and minimum parasitism was 76.57% at 20°C. These results also indicate that among two host eggs, greater parasitism was obtained

from *C. cephalonica* as compared to *P. xylostella* eggs at all temperatures. The most favourable temperature was 25°C. Further, results indicate that parasitism on both host eggs were significant to each other at same temperature.

Per cent adult emergence of *T. chilonis* on *P. xylostella* eggs

In the present studies, maximum adult *T. chilonis* emergence from *P. xylostella* parasitized eggs was 79.29% at 25 °C followed by 72.86% at 30 °C and 65.14% at 20 °C. Similarly, adult *T. chilonis* emergence on *C. cephalonica* eggs were 81.29% at 25°C followed by 69.43% at 30°C and minimum was 63.57% at 20°C. The results further indicate that among two host eggs, *P. xylostella* were significantly different from *C. cephalonica* eggs for adult *T. chilonis* emergence. Greater adult *T. chilonis* emergence was obtained from *C. cephalonica* eggs as compared to *P. xylostella* eggs at three temperatures and most favourable temperature was 25°C.

Adult longevity of *T. chilonis* on *P. xylostella* eggs

At 20°C, the adult longevity was recorded longest (4.57 days), followed by (3.71 days) at 25°C and least was recorded at 30°C (2.86 days) which is significant from other two temperatures. Naik et al. (2015) reported that significant difference was noticed in the longevity of parasitoid emerged from eggs of different hosts. However, Ara (2015) reported maximum adult longevity of *T. chilonis* in *C. cephalonica*, *H. armigera*, *S. litura* and *S. c. ricini* eggs at 28°C. Thus, the present study indicated that the temperature of 20°C was found best for adult longevity of *T. chilonis* on *P. xylostella* eggs among all the temperatures tested (Table 2).

Developmental period of *T. chilonis* on *P. xylostella* eggs

The development time of *T. chilonis* from egg to adult in *P. xylostella* eggs was observed highest (8.71 days) at 20°C, followed by (8.29 days) at 25°C and minimum was observed at 30°C (5.86 days). The developmental period of *T. chilonis* varied according to the host species. Development time needed by the *Trichogramma* strains was shorter as temperature increased. This finding is in agreement with Consoli & Parra (1995) who assumed that this might be due to a more

appropriate metabolic process of the immature stages. The optimal temperature for development and survival was 25°C (McDougall & Mills 1997). The effective heat units of *T. chilonis* for development in the DBM eggs were greater reported for the *E. kueeniella* eggs by Hirashima et al. (1990) which is in accordance with our present findings that development of *Trichogramma* adult took longer days in DBM eggs than *C. cephalonica* eggs. The difference in these values is thought to be attributable to the difference in the quality and/ or quality of contents of the DBM and *C. cephalonica* eggs. Pintureau and Bolland (2001) also founded that as temperature increases, the duration of development decreases. The developmental period of *T. chilonis* on DBM eggs was also reported by Saranthoy et al. (1989). Although the temperatures in their studies were slightly different from those in the present one, the developmental period of the parasitoid is apparently shorter than that of the DBM. The results indicate that temperature has a significant effect on the biological parameters of *T. chilonis* and with increasing temperature developmental duration decreased

Table 2. Percent parasitization, developmental period (egg to adult), emergence rate and adult longevity of *T. chilonis* on *P. xylostella* eggs at different temperatures

Temp (°C)	Parasitization capacity %	Developmental period (days)	Emergence rate %	Longevity period (days)
20	69.14 (56.26) ^a	8.71 ^b	65.14 (53.82) ^a	4.57 ^c
25	82.71 (65.46) ^c	8.29 ^b	79.29 (62.93) ^c	3.71 ^b
30	77.57 (61.80) ^b	5.86 ^a	72.86 (58.61) ^b	2.86 ^a
SEM (±)	0.66	0.17	0.37	0.18
SE(d)	0.94	0.24	0.53	0.25
LSD (P = 0.05)	1.97	0.51	1.11	0.53

Figures in parenthesis are angular transformation values

Value following different letter down the column are significantly different using Turkey's HSD test

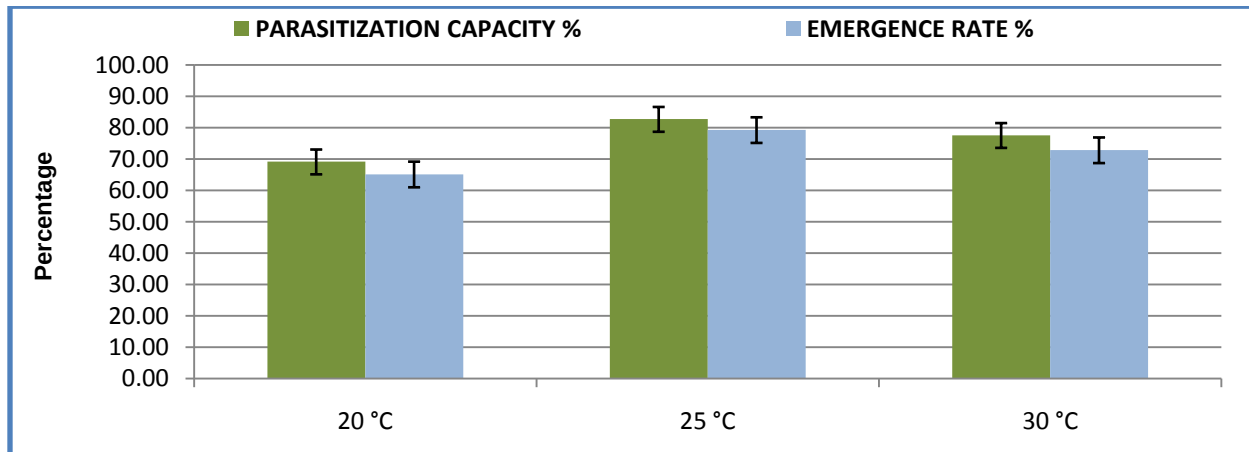


Figure 3. Graphical representation on parasitization and emergence rate of *T. chilonis* at different temperatures on *P. xylostella* eggs

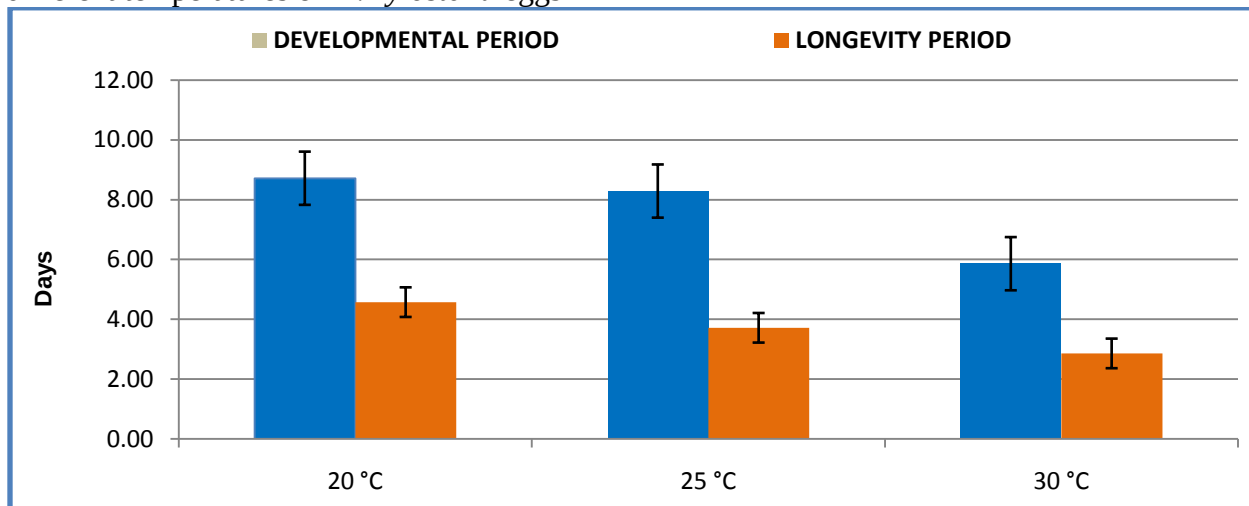


Figure 4. Graphical representation on developmental period and longevity period of *T. chilonis* at different temperatures on *P. xylostella* eggs

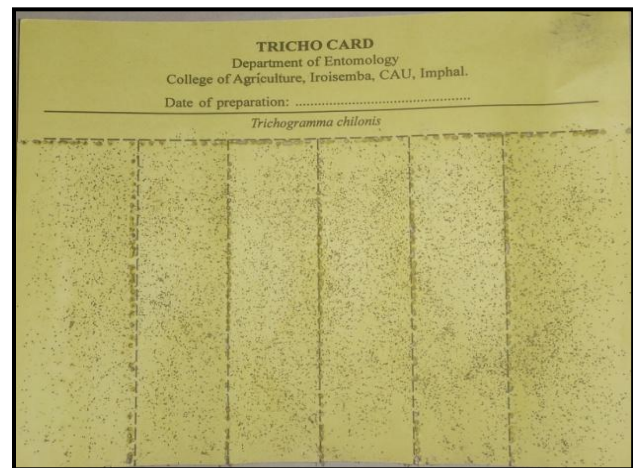
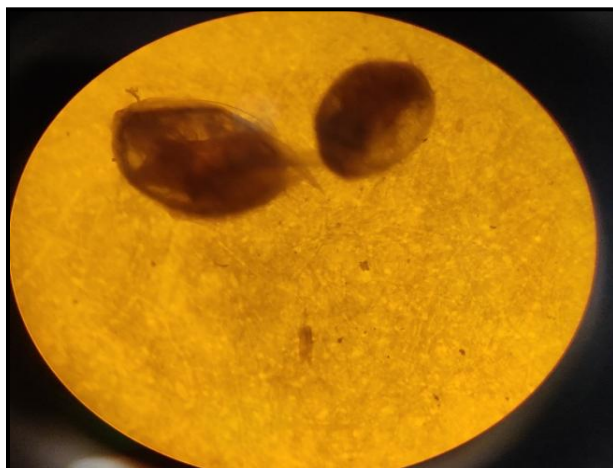


Plate 1. Parasitized *C. cephalonica* and *P. xylostella* eggs

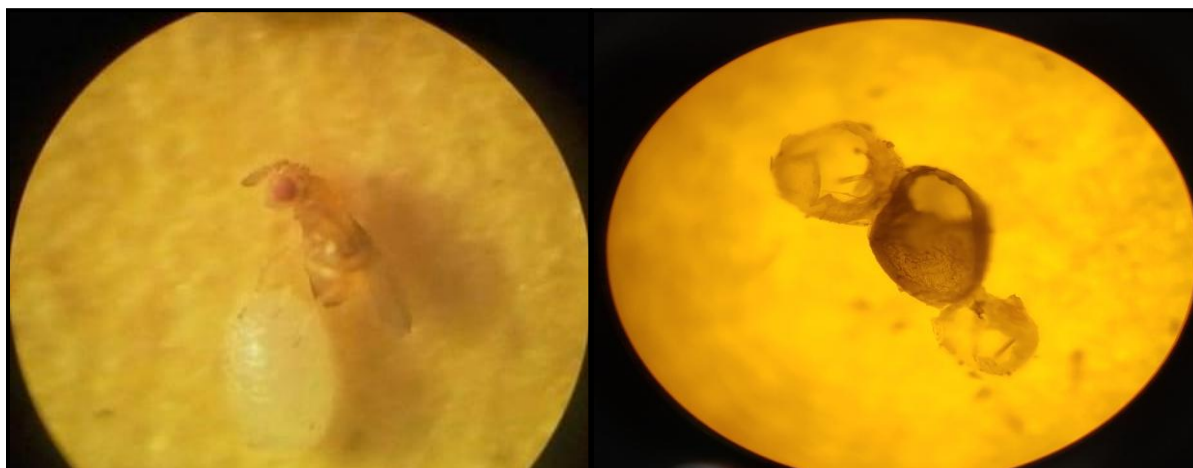


Plate 2. Eggs with hole of *T. chilonis* emergence and *T. chilonis* adult



Plate 3. Effect of biological parameters of *T. chilonis* on *C. cephalonica* and *P. xylostella* eggs at different temperature

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