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INFLUENCE OF LOW TEMPERATURE ON JUVENILE DEVELOPMENT, ADULT EMERGENCE AND PARASITISM OF TRICHOGRAMMA CHILONIS IN CAPTIVITY

[a]Altamash Khan, [b]Javid A. Abro, [b]Sher Ahmed*, [c]Zahoor A. Khetran, [a]Shahzad A. Shahwani, [b]Abid Hussain, [d]Muhammad Amin, [b]Abdul Latif, [e]Asif Mastoi, [b]Zaheer Ahmed

[a] Agriculture Research Institute ARI Sariab Quetta, Pakistan

[b] PARC-Balochistan Agricultural Research and Development Center Quetta, Pakistan

[c] PARC-Balochistan Agricultural Research and Development ARI, Jaffarabad, Pakistan

[d] Oil Seed department Agriculture Extension Rani Bagh Quetta, Pakistan

[e] Balochistan Agriculture Department Quetta, Pakistan

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ABSTRACT

The study was conducted in mass-rearing laboratories of beneficial insects at the Nuclear Institute of Agriculture Tandojam, to determine the optimum storage temperature of parasitoid Trichogramma chilonis after parasitization on Sitotroga cerealella eggs. Two hundred host eggs were pasted on white paper card strips with Vachellia nilotica tree gum, and these card strips were offered to parasitoid *T. chilonis* adults confined in glass jars. Honey solution (10%) on paper strips was provided to parasitoids as an adult diet inside the glass vessels. For parasitization, the host eggs on the strip, referred to as parasitized eggs, were taken out from glass jars and kept at six different low temperatures of 10, 8, 6, 4, -6 and -4°C along with control 25±2oC temperature in complete darkness. The results showed that the weekly maximum parasitism percentage of Trichogramma chilonis under lab conditions was observed at 10oC followed by 8oC and 6°C. The minimum parasitism percentage of Trichogramma chilonis under lab conditions was observed at 4°C. Weekly maximum adult emergence of Trichogramma chilonis under lab conditions was observed at 10°C followed by 8°C and 6°C. Minimum adult emergence of Trichogramma chilonis under lab conditions was observed at 4°C. The weekly maximum juvenile development period of Trichogramma chilonis under lab conditions was observed at 4°C followed by 6oC and 8oC. The minimum juvenile development period of Trichogramma chilonis under lab conditions was observed at 10°C. While no emergence was found in -6°C and -4°C. The developmental period was positively correlated with the storage period and the duration of development significantly decreases as the temperature increases from lower to higher concentrations.

Corresponding Author: Sher Ahmed

Email: shershahwani@parc.gov.pk

INTRODUCTION

Climate impacts have significantly enhanced the introduction and existence of many insect pests in new topographical areas, producing generous ecological and economic effects (Roques et al., 2016.) Thus, developing integrated pest management (IPM) programs must reduce the damage caused by these pests below the economic injury levels and consequently mitigate the use of insecticides (Del Pino et al., 2015). Amid these approaches, inundative biological control with egg parasitoids of the genus Trichogramma (Hymenoptera: Trichogrammatidae) has shown efficient results in controlling several lepidopteran pests in many crops and forests worldwide (Desneux et al., 2010, Mills, 2010). In the natural environment, insects experience fluctuating temperatures and evolved adaptations to extreme temperatures. To select the most efficient biological control program with Trichogramma, a strain selection is needed to efficiently use against target pests in natural given environmental conditions (Hassan, 2014). *T. chilonis* is made available throughout the season for storing and field release. By understanding the limits of *T. chilonis* seasonal release in the field from insectaries cultures. Mass rearing insectaries for field release and research required stock availability and adequate storage (Greenberg *et al*., 2014). Temperature directly influences parasitoid development during the developmental period, and previous researchers stated that the Trichogramma species' shelf life has been prolonged at low temperatures during storage duration to retain viability (Hoffmann *et al*., 2001). Kalyebi *et al*. (2006) studied egg parasitoids Trichogrammatid parasitism rate at different temperatures and relative humidity. Pitcher *et al*. (2002) reported *T. ostriniae* production method reared on moth eggs and stored at variable temperatures for eight weeks after parasitism. According to their study, emergence was completed in 2 weeks at 15^{o}C compared to 4 and 6 weeks at temperatures of 9 and 12oC, respectively. *T. ostriniae* stored at 6^{o}C gradually declined in emergence percentage. Attempts have been made to improve bio-control agents' effectiveness in reducing adverse climatic conditions. Temperature is essential, as it can affect the parasitic capabilities of egg parasitoids in abundance. The successful selection and introduction of *Trichogramma* wasps in biological control programs are determined by some critical factors, such as the potential development of the parasitoid species or strain in the target host, besides some abiotic and physical factors (De Freitas et al., 2012; Coelho et al., 2016; De Oliveira 2017). Adult parasitoids, but their impacts on immature stages are often ignored. Using Trichogramma wasps as a biocontrol agent in India is widely promoted for agricultural pests (Firake and Khan, 2014). This study aims to evaluate the parasitism rate of egg parasitoid *Trichogramma chilonis* at low temperatures during storage in the refrigerator, which was helpful in seasonal crop release.

MATERIALS AND METHODS

The present study was conducted in mass-rearing laboratories of beneficial insects at the Nuclear Institute of Agriculture Tandojam to determine the optimum storage temperature for parasitoid, *Trichogramma chilonis under* laboratory conditions. The following steps were adopted to conduct this study.

Host, Sitotroga cerealella

The Angioumos grain moth (rice grain moth), *S cerealella* was reared under a bio-control laboratory Nuclear Institute of Agriculture (NIA) as a factitious host of egg, parasitoid, *Trichogramma chilonis* under laboratory temperature 27±2 ^{o}C with relative humidity (RH) 65%. S*. cerealella* was reared on the wheat mixture in the laboratory; about 20 kg of wheat was treated daily at 100 ^{o}C boiling point for 10 minutes to prevent contamination of predatory mites and other fungal diseases. The treated wheat mixture was kept in sunlight for 24 hours to maintain the moisture of the grain; when wheat grains were ready to use, about 750 grams were kept in 4-litre glass jars. Then *S.cerealella* eggs, about 5 ml were scattered on wheat mixture inside the glass jars and covered with muslin cloth. When adults emerged in glass jars after 24-25 days, they were collected through electric suction pump daily. The emerging moth was shifted into an egg-laying device made up of a plastic jar about 3" in size; the bottom of the device was removed to paste a nylon net 40 mesh in size with a second bond for egg-laying purposes. These egg-laying devices were sieved daily with a sieved device made of 80 mesh iron net to obtain eggs for rearing of egg parasitoid *T.chilonis*.

Egg parasitoid, Trichogramma chilonis

T. chilonis adults were obtained from culture reared in a bio-control laboratory at 26±2 ^{o}C with relative humidity (RH) of 50%. For experimentation, adults of *T.chilonis* were identified with sterenomicroscope for differentiation in males and females through antennae. Identified adults were paired in jam glass jars, with ten pairs each. Honey solution (10%) on the paper strip was provided to parasitoids as an adult diet inside the glass vessels daily. Two hundred (200) eggs were pasted on white paper card strips with Vachellia *nilotica* tree gum; these card strips were offered to parasitoid, *T. chilonis* adults inside the glass jars for parasitization for 24 hours.

After 24 hours, these parasitized strips were removed from the glass jars and kept at six different low-temperature regimes i.e; 10, 8, 6, 4, -6 and -4°C, confined in different incubators to determine the optimum storing temperature and duration. The examined parameters for the experiment were:

Juvenile development

Trichogramma chilonis is an endoparasite egg parasitoid; the larval duration of *T.chilonis* was recorded inside the incubators through placed egg card strips. Before keeping fresh egg card strips in incubators were brown at different low temperatures, the hatched larvae of *T. chilonis* developed slowly inside the host egg. When these eggs were blackish, this duration was recorded for the juvenile development period / larval duration of *T.chilonis.*

Parasitization percentage (%)

The parasitization percentage of adult females of *T.chilonis* was recorded based on host eggs being visible blackish on different temperature regimes confined in different incubators. The number of blackish-coloured eggs was counted on a sterenomicroscope and recorded as a parasitization percentage (%) parameter.

Adult emergence

Two methods recorded the number of adult emergences of T. chilonis after 6-7 days of emergence time. One counts the hole in host eggs with a stereno microscope, and the second counts with several adults who emerged kept at different temperature regimes confined in incubators.

RESULTS

Juvenile development period (in days)

The results revealed low-temperature effects on juvenile development period of Trichogramma chilonis under lab conditions, as shown in Table 1. The data indicates a significant difference in juvenile development period between the temperatures, weeks and their interactions. During 1st week, the maximum juvenile development period of *T. chilonis* (20.00±0.00) in days was recorded at 4°C followed by (18.00±2.65) at 6°C and (16.00±1.00) at 8°C. The minimum juvenile development period (14.00±3.61) in days was recorded at 10°C. During 2nd week, the maximum juvenile development period of *T. chilonis* (22.00±0.00) in days was recorded at 4°C followed by (20.00±0.00) at 6°C and (18.00±1.73) at 8°C. The minimum juvenile development period (16.00±1.00) was recorded at 10⁰C. During 3rd week, the maximum juvenile development period of *T. chilonis* (23.66±1.53) in days was recorded at 4°C followed by (22.00±0.00) at 6°C and (20.00±0.0) 8°C. The minimum juvenile development period (18.00±1.73) was recorded at 10°C. During the 4th week, the maximum juvenile development period of *T. chilonis* (25.66±0.58) in days was recorded at 4°C followed by (24.00±1.00) at 6°C and (22.00±0.00) at 8°C. At the same time, the minimum juvenile development period (20.00±0.00) was recorded at 10°C. The results showed that the most extended juvenile development period of T. chilonis was observed at 4oC followed by 6oC and 8oC, while the shortest juvenile development period was determined at 10oC (Figure 1).

Table 1. Effect of low temperature on juvenile development period (in days) of *Trichogramma chilonis* under lab conditions.

Temperature (°C)	Weeks			
	1st	2nd	3rd	4th
10°C	14.00±3.61g	16.00±1.00f	18.00±1.73e	20.00±0.00d
8°C	16.00±1.00f	18.00±1.73e	20.00±0.00d	22.00±0.00c
6°C	18.00±2.65e	20.00±0.00d	22.00±0.00c	24.00±1.00ab
4°C	20.00±0.00d	22.00±0.00c	23.66±1.53bc	25.66±0.58a
-6°C	------	-----	----	-----
-4°C	---	-----	----	----

Parasitism percentage (%) of *Trichogramma chilonis*

The results of this study on the effect of different low-temperature regimes on parasitism percentage (%) of *T.chilonis* are shown in Table 2. The data indicates a significant difference in parasitism percentage between the temperatures, weeks and their interactions. During

1st week, the maximum parasitism percentage (73.33±1.53) of *T. chilonis* was recorded at 10ºC followed by (70.66±1.15) at 8ºC and (61.66±1.53) at 6ºC. However, the minimum parasitism percentage (49.33±2.08) was recorded at 4⁰C. During 2nd week, the the maximum parasitism percentage of *T. chilonis* (68.66±3.21) was recorded at 10ºC followed by (68.33±3.51) at 8ºC and (56.33±1.53) at 6ºC. In contrast, the minimum parasitism percentage (45.66±3.06) was recorded at 4ºC. During 3rd week, the maximum parasitism percentage of *T. chilonis* (46.33±4.73) was recorded at 10ºC followed by (42.66±1.53) at 8ºC and (43.00±1.73) at 6ºC. whereas, parasitism % (20.66±2.08) was recorded at 4ºC. During 4th week, the maximum parasitism percentage of *T. chilonis* (44.33±2.08) was recorded at 10ºC followed by (35.33±2.08) at 8ºC and (27.00±4.00) at 6ºC. Similarly, a minimum parasitism percentage of (8.33±2.52) was recorded at 4ºC. The results further showed that a higher parasitism % of *T. chilonis* was observed at 10ºC followed by 8ºC and 6ºC. The lowest parasitism percentage was determined at 4ºC, as shown in (Figure 2).

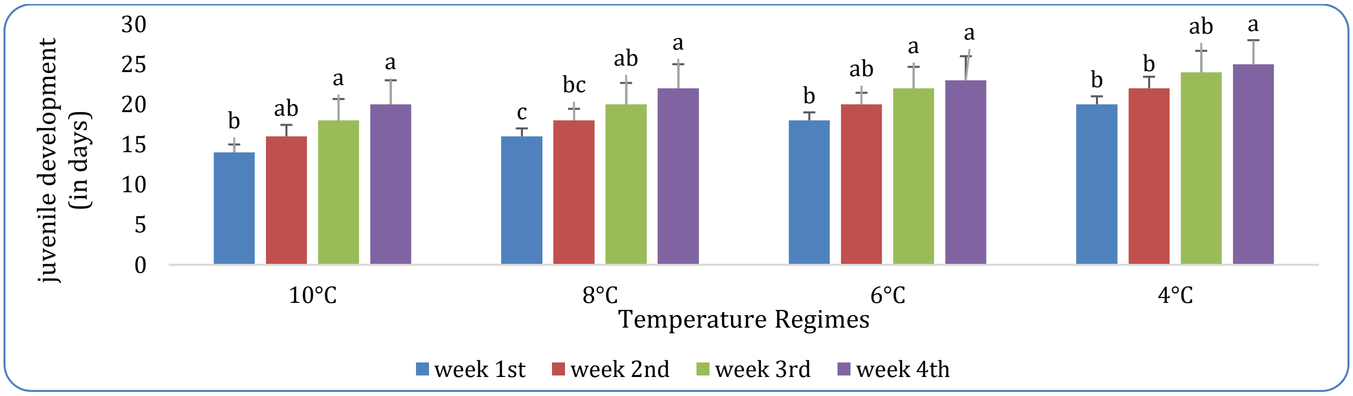

Figure 1. Effect of different low-temperature regimes on juvenile development of T. chilonis.

Table 2. Effect of low temperature on parasitism percentage of *Trichogramma chilonis* under lab conditions.

Temperature (ºC)	Weeks			
	1st	2nd	3rd	4th
10ºC	73.33±1.53a	68.66±3.21b	46.33±4.73ef	44.33±2.08f
8ºC	70.66±1.15ab	68.33±3.51b	42.66±1.53f	35.33±2.08g
6ºC	61.66±1.53c	56.33±1.53d	43.00±1.73f	27.00±4.00h
4ºC	49.33±2.08e	45.66±3.06ef	20.66±2.08i	8.33±2.52j
-6ºC	----------------	------------------	----------	------------------
-4ºC	------------------	------------------	---------------	----------------

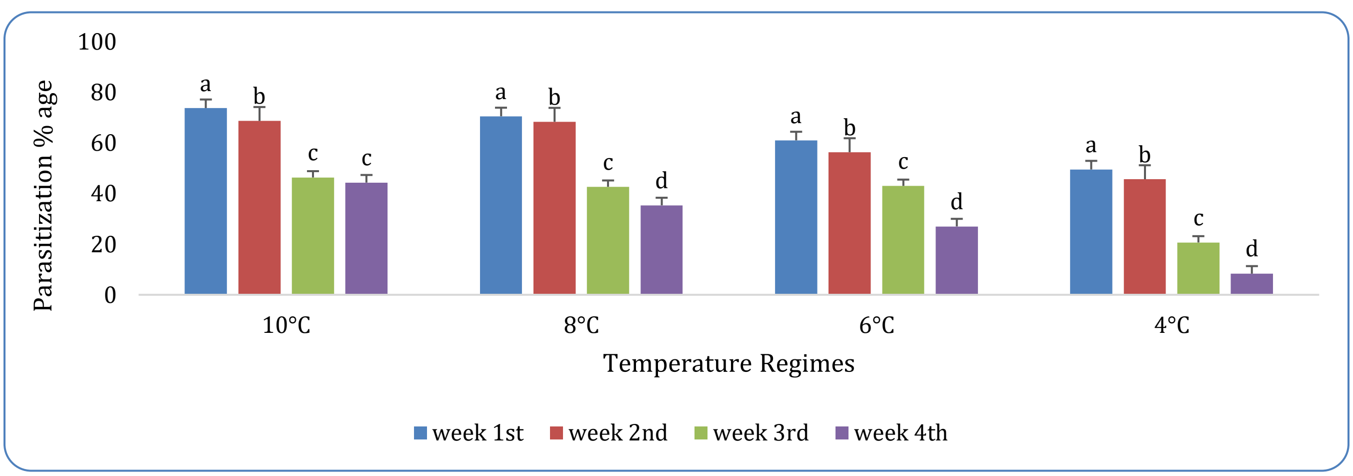

Figure 2. Effect of different low-temperature regimes on parasitization percentage of *T. chilonis*.

Adult emergence of Trichogramma chilonis

The present study's results revealed the effect of different low-temperature regimes on the adult emergence of *T. chilonis* during storage, presented in (Table 3). The data indicates a significant difference in adult emergence between the temperatures and weeks and non-significant for their interactions. During 1st week, the maximum number of *T. chilonis* adult emergence (20.00±5.00) was recorded at 10ºC followed by (19.33±5.03) at 8ºC and (16.66±3.06) at 6ºC. In comparison, a minimum number of adult emergence (9.33±3.06) was recorded at 4ºC. During 2nd week, maximum number of *T. chilonis* adult emergence (23.66±5.13) was recorded at 10ºC followed by (23.33±2.89) at 8ºC and (15.33±3.06) at 6ºC. Although, the minimum number of adult emergence (11.33±3.21) was recorded at 4ºC. During 3rd week, a maximum number of *T. chilonis* adult emergence (23.66±3.21) was recorded at 10ºC followed by (17.00±4.36) at 8ºC and (16.00±4.00) at 6ºC. Similarly, a minimum number of adult emergence (14.66±5.03) was recorded at 4ºC. During 4th week, a maximum number of *T. chilonis* adult emergence (26.66±1.53) was recorded at 10ºC followed by (21.66±4.73) at 8ºC and (19.00±6.56) at 6ºC. Moreover, a minimum number of adult emergence (17.66±2.52) was recorded at 4ºC. The results further showed that higher adult emergence of *T. chilonis* was observed at 10ºC followed by 8ºC and 6ºC, while the lowest adult emergence was determined at 4ºC presented in Figure 3.

Table 3. Effect of low temperature on adult emergence percentage of Trichogramma chilonis under lab conditions.

Temperature (ºC)	Weeks			
	1st	2nd	3rd	4th
10ºC	20.00±5.00 bcd	23.66±5.13 ab	23.66±3.21 ab	26.66±1.53 a
8ºC	19.33±5.03 bcd	23.33±2.89 ab	17.00±4.36 cd	21.66±4.73 abc
6ºC	16.66±3.06 cde	15.33±3.06 de	16.00±4.00 de	19.00±6.56 bcd
4ºC	9.33±3.06 f	11.33±3.21 ef	14.66±5.03 def	17.66±2.52 cd
-6ºC	-------	-------	-------	------
-4ºC	-------	-------	--------	-------

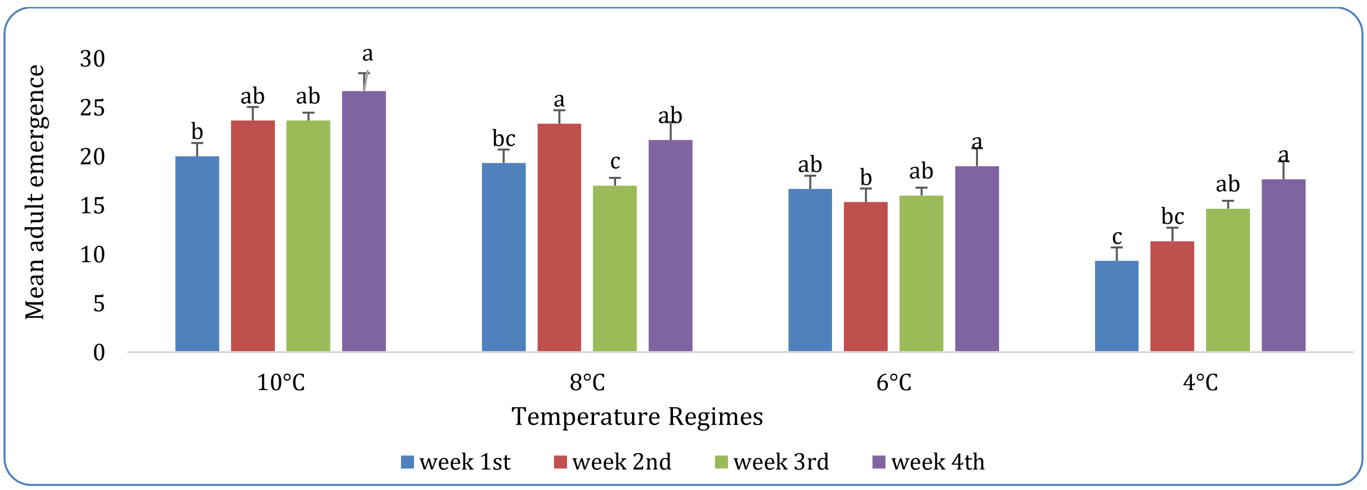

Figure 3. Effect of low temperature regimes on number of adult emergences of *T. chilonis.*

DISCUSSION

Our study observed the weekly maximum parasitism percentage of Trichogramma chilonis under lab conditions at 10ºC followed by 8ºC and 6ºC. The minimum parasitism percentage of *Trichogramma chilonis* under lab conditions was observed at 4ºC. These results follow the findings of Pitcher et al. (2002), who reported *T. ostriniae* production method was reared on moth eggs and stored at variable temperatures for eight weeks after parasitism. According to their study, emergence was completed in 2 weeks at 15ºC compared to 4 and 6 weeks at temperatures of 9 and 12ºC, respectively. *T. ostriniae* stored at 6ºC gradually declined in emergence percentage. In comparison to the control, the rate of parasitism was similar after 2 to 4 weeks of storage at temperatures of 9 and 12ºC, while the decline in parasitism percent due to inclined in storage period greater than 4 weeks was observed, and the most suitable temperature for storage was 9ºC. Nadeem et al. (2010) studied the response of *T. chilonis* under low-temperature regimes. Their findings stated at 10ºC, the parasitism rate was considerably higher (97.4%) after being kept for five days of storage afterwards, the parasitism rate eventually decreased up to 42.2% at the same temperature after 90 days of storage. Firake and Khan (2014) stated the *Trichogramma* spp. It can be reared under lower temperatures beyond 16°C to extend the longevity and parasitism percentage under field conditions. Vigneswaran et al. (2017) revealed that at 10°C, the highest parasitism percentage (96.00%) was observed and further decreased to 53.66% at 10°C when stored for up to 30 days. In contrast, the lowest parasitism percentage was assessed at 16 (89.83%) and 6°C (90.03%). In our weekly study, maximum adult emergence of *Trichogramma chilonis* under lab conditions was observed at 10ºC followed by 8ºC and 6ºC.

Minimum adult emergence of *Trichogramma chilonis* under lab conditions was observed at 4ºC. Young et al. (2000) observed a maximum emergence rate (88.4%) at 28ºC followed by 78 and 60.7% at 26 and 30ºC, whereas a minimum emergence rate (50.1%) was recorded under 32ºC. Mehendale (2009) their findings revealed that adult emergence of 89.0% was observed at 5 days storage than 75.0%, 79.31%, 68.47%, 63.97% and 63.80% on 15-, 20-, 25- and 25-days storage and afterwards, the emergence drastically declined. He concluded that 5°C temperature was effective for parasitized trichocards. Nadeem et al*.* (2010) studied the response of *T. chilonis* under low-temperature regimes. Their findings stated that the maximum emergence of *T. chilonis* (96.6%) was recorded at 10°C after five days of storage, and the same emergence percentage of *T. chilonis* was observed for control (97.4%). Vigneswaran et al*.* (2017) revealed that 10°C significantly increased. In our study, the weekly maximum juvenile development period of *Trichogramma chilonis* under lab conditions was observed at 4⁰C followed by 6⁰C and 8ºC. The minimum juvenile development period of *Trichogramma chilonis* under lab conditions was observed at 10ºC. Young et al*.* (2000) their results revealed that the minimum development time (egg-adult) was 8.6 days at 32ºC followed by 9.0 and 9.6 days at 30 and 28ºC, and the maximum development time (egg-adult) was 10.9 days at 26ºC for T. *dendrolimi*. Kalyebi et al*.* (2006) revealed that at 10°C, both species had no development and parasitoids were somewhat developed. At 2°C, the longest development time was recorded for *Trichogramma* spp. And the shortest development time was noted for *Trichogrammatoidea*. Sergey et al. (2009) stated that the temperatures significantly influence the development and growth rate of insects. Ahmad *et al.* (2011) state that the developmental period is positively correlated with the storage period. Bari *et al.* (2015) state that the duration of development significantly decreases as the temperature increases from lower to higher concentrations.

CONCLUSION

Storage temperature of parasitoid, Trichogramma chilonis after parasitization on Sitotroga cerealella eggs. Two hundred host eggs were pasted on white paper card strips with Vachellia nilotica tree gum; these cards were offered to parasitoid T. chilonis adults confined in glass jars. At six different low temperatures of 10, 8, 6, 4, -6 and -4°C, control 25±2oC temperature in complete darkness. The weekly maximum parasitism percentage of Trichogramma chilonis under lab conditions was observed at 10oC followed by 8oC and 6oC. The minimum parasitism percentage of Trichogramma chilonis under lab conditions was observed at 4oC. Weekly maximum adult emergence of Trichogramma chilonis under lab conditions was observed at 10oC followed by 8oC and 6oC. Minimum adult emergence of Trichogramma chilonis under lab conditions was observed at 4oC. The present study can help farmers, stakeholders and students know mass-rearing techniques in laboratory conditions of Trichogramma chilonis from egg to adult to enhance the biological control of different Agricultural pest species.

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