

Potential for Mass Rearing of the Egg Parasitoids, *Trichogramma chilonis* and *Tricogramma achaeae* (Hymenoptera: Trichogrammatidae) on *Corcyra cephalonica* Eggs

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ABSTRACT: *Trichogramma chilonis* and *T. achaeae* are egg parasitoids, considered as ideal candidates for managing the cabbage looper (*Trichoplusia ni*) through augmentative release. Mass rearing with steady supply of parasitoid is necessary to promote augmentative release. Numbers of factors contribute to the steady supply of parasitoids. *Corcyra cephalonica* eggs are extensively used for mass rearing. When there is an excess supply of host eggs, those eggs should be stored and used for parasitization when needs arise. Further, steady supply of parasitoids can be assured if parasitized eggs can be stored when there is excess supply. Adult parasitoids should be maintained as a parent stock and they should be fed with suitable food at the right concentration using a suitable feeding technique. In addition, it is important to know how many generations of parasitoids can be run continuously on *C. cephalonica* eggs without losing the parasitization capacity. These aspects were studied with the objective of improving the existing mass rearing protocol. *T. achaeae* and *T. chilonis* accepted stored eggs for parasitization, but the level of acceptance significantly varied with storage durations. The *C. cephalonica* eggs stored for two weeks at 4 or 8 °C were acceptable for parasitization. Pupal stage of both parasitoids within parasitized eggs can be stored upto two weeks at 4 °C or four weeks at 8 °C while maintaining at least 70% adult emergence. Performance of emerging adults in terms of parasitism significantly varied with storage duration. Bee honey was a better type of food source compared with glucose, fructose and sucrose for feeding parasitoids. The parasitoid adults can be fed with 50% bee honey using drop method successfully. Both species responded differently for number of generations that can be run on *C. cephalonica* eggs. It was four generations for *T. achaeae* and eight for *T. chilonis*.

Keywords: Dispersal, mass rearing, parasitism, *Trichogramma*

INTRODUCTION

Trichogramma species are widely used egg parasitoids for biological control of insect pests of different crops through augmentation and release. More than 16 million ha of crops are treated with *Trichogramma* species annually (van Lenteren, 2000). *Trichogramma chilonis* and *T. achaeae* have been promoted to use in controlling cabbage leaf eating caterpillars (Krishnamoorthy, 2012). In this process, a steady production or supply of the egg parasitoids

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is essential to achieve successful control. However, production process could fluctuate due to many reasons, especially the availability of host eggs for parasitization and parent stock of parasitoids for parasitization, maintenance of adult parasitoids on artificial diet, and maintaining the quality of adults when they are under continuous rearing on surrogate hosts.

Production of *Corcyra cephalonica* is done using crushed corn and the egg supply varies with the quality of the parent culture (Bigler, 1994; Greenberg *et al.*, 1996). There is a variation in the production of eggs with time due to the biological nature of the material, which subsequently affect the production cycle of *Trichogramma* species. This could be smoothened if the *C. cephalonica* eggs can be stored in cool temperature arresting the development of embryo within egg (Sithanatham *et al.*, 2013). Stored eggs can be used in *Trichogramma* production when the need arises. Absence of scientifically valid data on this aspect warrants investigation. It is of primary importance to find the suitable temperature and duration for host-egg storage without affecting the acceptance of *T. achaeae* or *T. chilonis* for parasitization.

The adult parasitoids should also be available on time when the host eggs are available for smooth production of *Trichogramma* species. In addition, the demand for *Trichogramma* species may vary with the number of farmers interested on this approach, level of pest attack in the field and stage of the crop, etc. Fluctuating and variable demand for the adult parasitoid could be met if the parasitoids can be stored at their pupal stage of development under cool temperature (Malik, 2001). However, information on this aspect with respect to *T. achaeae* and *T. chilonis* is not available and hence demands investigation, *as it* is important to find the suitable temperature for the storage of parasitoid pupa without affecting adult emergence and the related mortality.

Moreover, the parent stock of adult parasitoid should be able to maintain on an artificial diet, probably on a sugar solution until they have an access to natural food sources in the field (Romeis *et al.*, 2005). The data on this aspect is also scanty with respect to *T. achaeae* and *T. chilonis*, and requires investigation especially focussing on the type of sugar sources and the method of feeding suitable for the adult parasitoid.

The loss of quality of the parent parasitoid culture when the adults are being continuously reared on a surrogate hosts is well documented (Bigler, 1994; Greenberg *et al.*, 1996). Quality loss could be associated with the ability of responding parasitoid to chemical cues, which eventually affects the searching ability of host eggs for parasitization. With meagre information on this aspect in relation to *T. achaeae* and *T. chilonis*, investigation on the number of generations that can be reared on *C. cephalonica* eggs without affecting the capacity for parasitization is important.

As stated above, there is a demand for information in relation to the mass rearing of *T. chilonis* and *T. achaeae* for successful implementation of bio control programmes. Hence, this study was conducted with the objectives of examination of (a) potential for use of stored *C. cephalonica* eggs for *Trichogramma* production, (b) potential for storage of *Trichogramma* pupa in cool temperature, (c) suitability of four different sugar sources and their concentrations as food for adult parasitoids and (d) the number of *Trichogramma* generations that could be reared continuously on an alternate host *C. cephalonica*, without affecting the parasitization capacity.

METHODOLOGY

This study was conducted during 2013 at the Department of Agricultural Biology of the Faculty of Agriculture, University of Peradeniya, Sri Lanka.

(a) Potential use of cool-stored *C. cephalonica* eggs for production of *T. chilonis* and *T. achaeae*

Corcyra cephalonica eggs were used as the proven alternate host for the rearing of *T. achaeae* and *T. chilonis*. Egg cards were prepared by using 100, one-day-old eggs of *C. cephalonica* on 3 cm x 2 cm A4 paper using a thin layer of ordinary glue. These egg cards were stored as batches (ten egg cards per batch) under two different temperatures, i.e. 4 °C and 8 °C. The temperatures were selected based on the previous work of Nadeem *et al.* (2010). Every week, a fresh batch of egg cards was added to the stock. After seven weeks, all the egg cards were taken out at once and used for assessing the acceptance for parasitization by the parasitoid adult. At this point, there were eight batches with the storage durations of 0, 1, 2, 3, 4, 5, 6 and 7 weeks. Ten egg cards in each batch were placed in 5.5 cm tall, 4.3 cm diameter clear plastic vials separately. Two newly emerged female insects of *T. achaeae* were introduced to each vial allowing them to parasitize the eggs on egg cards. Diluted honey was supplied as food for the parasitoids. After six days, the egg cards were examined under a dissecting microscope (10 x 4, Meiji, Japan) to determine the parasitization. The parasitized eggs could easily be detected as they turn black due to the developing immature stage of *Trichogramma* spp. inside the egg. The level of parasitism was recorded in each egg cards that were stored for different durations under two temperatures. The same procedure was followed using *T. chilonis*.

(b) Potential for storing parasitized eggs at 4 °C and 8 °C

Egg cards were prepared using one-day-old *C. cephalonica* eggs glued to 3 cm x 2 cm paper. The egg cards containing 100 eggs per card were separately exposed to five females of *T. chilonis* and *T. achaeae* for parasitization within a clear plastic vial (5.5 cm height and 4.3 cm diameter). Six days after parasitization, the egg cards were cut into small pieces containing 30 eggs/card. At this time, immature *T. chilonis* has reached the pupal stage (Hussain *et al.*, 2012). These small parasitized egg cards were stored under 4 °C and 8 °C, as suggested by Malik (2001) over different durations up to three months. A batch of ten stored egg cards was taken out at every week, and warmed to the room temperature, and incubated until the adult emergence. In this process, there were 12 egg batches with 12 stored durations varying from 1 to 12 weeks. After emergence of adult parasitoids, level of emergence of parasitoids from each egg card was recorded.

The quality (performance) of parasitoid adults emerging from eggs that had been stored for different durations were assessed using two females that were randomly obtained from each particular stored duration (total of 20 females). They were given fresh *C. cephalonica* eggs cards (100 eggs per card) and the level of parasitism was used as the criterion for assessing the adult performance. This procedure was separately carried out for both *T. achaeae* and *T. chilonis*. Level of parasitism was calculated using the following equation given by Van Driesche (1983).

$$\text{Level of parasitism} = \frac{\text{No. of adult parasitoids}}{\text{No. of adult parasitoids} + \text{No. of unparasitized eggs}} \times 100$$

(c) Assessment of suitable food for adult *T. chilonis*

Sources of food

Different sugars were evaluated for their suitability as a food source in terms of adult longevity in comparison with parasitoids fed with distilled water and starved parasitoids. The selected sugar sources for this study were glucose, fructose, sucrose and undiluted bee honey. These food sources were used as saturated solutions. Foods were introduced by placing a drop of each sugar solution on inner side of the lid of the plastic vials. Newly emerged ten parasitoid adults were enclosed in a clear plastic vial (4.3 cm diameter and 5.5 cm height). Food source was replaced as needed. Vials were examined every day and the mortality and the longevity were recorded (Saljoqi and Khajjak, 2007).

Suitable concentration of bee honey

Bee honey was found as the most suitable food for parasitoid in the previous experiment. A series of concentrations of bee honey solutions were prepared, i.e. 100%, 50%, 33%, 25%, 20% and 16% by adding distilled water, with 0% (distilled water) as the control. Parasitoid adults were fed with these honey solutions using a filter paper when the adults were in clear plastic vials. Each vial (replicate) contained newly emerged 10 adults of the same age. There were ten replicates for each food concentration (100 adults). Suitability of the food source was evaluated by assessing the longevity of the adults. The method was adapted from Azzouz *et al.* (2004).

Evaluation of different feeding methods of adult parasitoids

Commonly used parasitoid adult feeding methods, namely filter paper, wick, sponge and drop methods, were evaluated. Concentrated bee honey was used as the food source for adult parasitoids. Filter paper (1/16 part of 8 cm diameter filter paper), wick (1.5 cm long) and sponge (1 x 1 x 1 cm) were submerged in concentrated bee honey solution for 3 min and excess honey was allowed to drain for 10 min. Food source in different methods were placed separately in clear plastic vials (4.3 cm and 5.5 cm height) containing 10, one day-old, parasitoids. In the drop method, one drop of each solution was placed on the bottom of separate transparent plastic containers. The control consisted of parasitoids with no food within the vial. The rearing containers were examined for mortality of parasitoids in the morning daily until the last adult parasitoid died.

(d) Potential for continuous rearing of *T. chilonis* and *T. achaeae* on *C. cephalonica* eggs

The quality of parasitoids in terms of parasitization capacity decreases when the adults were reared on alternative hosts continuously (Hopper *et al.*, 1993). To examine this fact, *T. chilonis* and *T. achaeae* adults were collected from *T. ni* eggs in cabbage fields at Thalatuoya area. The field collected eggs were individually placed in clear plastic vials and maintained at room temperature. Vials were examined daily for adult emergence and emerged adults were fed with bee honey solution. Eggs were prepared as egg cards similar to the previous experiments. Parasitized egg cards were incubated until the adult emergence and the emerging adults were provided with fresh egg cards separately within a clear plastic vial for parasitization. In each generation, the number of parasitized eggs was calculated examining the eggs under a dissecting microscope. The level of parasitism was calculated. Parasitoids were reared on *C. cephalonica* eggs continuously over 10 generations.

Data analysis

Levels of parasitism and per cent adult emergence were analyzed using ANOVA and the mean separation was done by using Tukey test. Count data were analyzed by the Chi-square test using Systat ver 11 computer software package.

RESULTS AND DISCUSSION

(a) Potential use of cool stored *C. cephalonica* eggs for production of *T. chilonis* and *T. achaeae*

Parasitoid adults accepted cool-stored eggs as a suitable host for parasitization. There were significant differences of acceptance between parasitoid species ($F=42.3$, $df=1$; 288 $p<0.001$); between temperatures ($F=4.5$, $df=1$; 288 $p=0.034$), among durations ($F=286$ $df=7$; 288 $p<0.001$). The interactions: species x temperature, temperature x duration and species x temperature x durations, were not significantly different ($p>0.05$). However, the interaction: species x durations ($F=3.4$, $df=7$; 288 $p=0.002$), was significantly different.

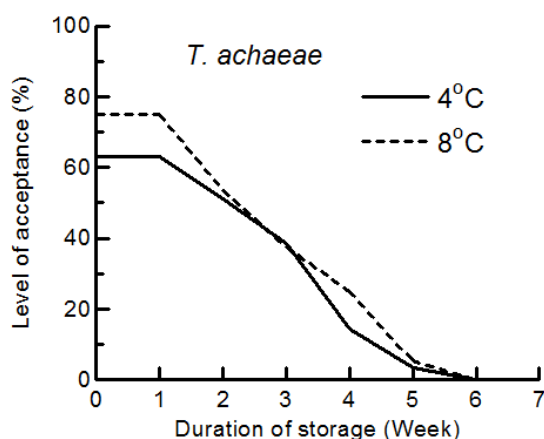


Fig. 1. Acceptance of *C. cephalonica* eggs stored at 4 and 8 °C for different durations by *T. achaeae* for parasitization.

With respect to *T. achaeae*, the mean egg acceptance ranged from $63.5 \pm 7.5\%$ at 0 week (no storage) to $3.3 \pm 1.1\%$ at 5th week at 4 °C. Eggs stored for 6 weeks or longer were not accepted (Fig. 1). At 8 °C the mean egg acceptance percentage of *T. achaeae* varied from 75.1 ± 4.9 at 0 week (no storage) to 5.4 ± 1.7 at 5th week. Eggs stored for 6 weeks or longer were not accepted (Fig. 1). It appears that host eggs can be stored up to two weeks at 4 °C and 8 °C and use for mass production of *T. achaeae*, where further storage may not be economical as the level of acceptance drops below 50%.

With respect to *T. chilonis*, the mean egg acceptance ranged from $83.5 \pm 3.2\%$ at 0 week (no storage) to $3.3 \pm 1.1\%$ at 5th week at 4 °C. The eggs stored for 6 weeks or longer were not accepted (Fig. 2). At 8 °C, the mean egg acceptance percentage of *T. chilonis* varied from 90.5 ± 1.9 at 0 week (no storage) to 1.0 ± 0.5 at 6th week. No acceptance of eggs stored for 7 weeks or longer (Fig. 2). Similar to the results observed in *T. achaea*, it appears that host

eggs can be stored up to two weeks and used for mass production *T. chilonis*, where further storage reduced acceptance to below 50% thus making the process not economical.

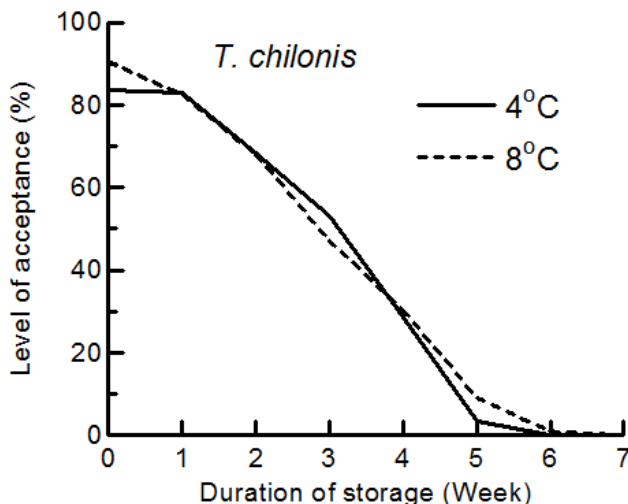


Fig. 2. Acceptance of *C. cephalonica* eggs stored at 4 and 8 °C for different durations by *T. chilonis* for parasitization

The acceptance of stored eggs may be associated with the survival of *C. cephalonica* embryo. Further, storage beyond 2 weeks may not be economical for both species as the parasitism percentage drops drastically with the storage time. However, two weeks of storage may be sufficient to carry out a smooth production cycle as any minor errors of the parent culture of *C. cephalonica* can be fixed within two weeks time.

(b) Potential for storing parasitized eggs at 4 and 8 °C

There was no significant difference in per cent adult emergence between the species *T. achaeae* and *T. chionis*. However, the percent adult emergence significantly differed under different temperatures ($F=577$, $df=1;260$ $p<0.001$) and storage durations ($F=832$, $df=12;260$ $P<0.001$). The interactions between species and temperature ($F=8$, $df=12;260$ $p=0.005$) and between temperature and duration ($F=16.6$, $df=12;260$ $p<0.001$) were also significantly different. However, the interactions species x durations and species x temperature x duration were not statistically significant ($p>0.05$).

At 4 °C, the adult emergence significantly varied with the storage duration ($F=521$, $df=12;143$ $p<0.001$). It varied from $93.3\pm 3.2\%$ (0 week storage) to $0.5\pm 0.5\%$ at the 9th week (Fig. 3). Similarly at 8 °C, the adult emergence significantly varied with the storage duration ($F=357$ $df=12;143$ $p<0.001$). It varied from $93.3\pm 3.2\%$ (0 week storage) to $0.25\pm 0.25\%$ at the 12th week (Fig. 3).

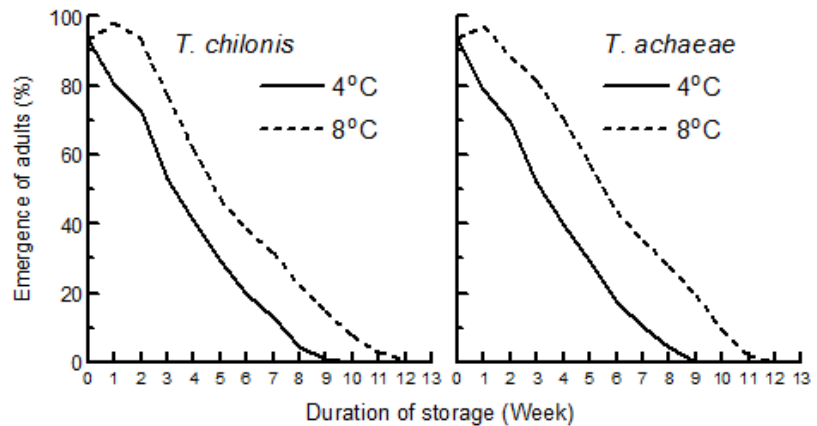


Fig. 3. Emergence of adult parasitoids from the parasitized host eggs that had been stored over different durations at 4 and 8 °C

These results indicated that the parasitized eggs can be stored for a longer period at 8 °C than at 4 °C. The economical storage time would be 4 weeks at 8 °C, retaining about 65% adult emergence while it was two weeks at 4 °C with about 70% adult emergence. Storage in cooler temperature (4 °C) might be more damaging to the pupa of both species of *Trichogramma* compared to at 8 °C. These results are useful in deciding the suitable stage for transportation and acceptable transporting duration when the parasitoids are delivered to the customers.

Parasitism of emerged parasitoids

There were significant differences of parasitism between parasitoid species ($F=533$, $df=1;572$ $p<0.001$); between temperatures ($F=178$, $df=1;572$ $p<0.001$), among durations ($F=916$, $df=12;572$ $p<0.001$). In addition, the interactions between species and temperature ($F=8.2$ $df=1;572$ $p<0.001$), species and durations ($F=17.3$, $df=12;572$ $p<0.001$), temperature and duration ($F=8.2$, $df=12;572$ $p<0.001$) and species x temperature x durations ($F=2$, $df=12;572$ $p<0.001$) were significantly different.

With respect to *T. achaeae*, the level of parasitism significantly varied between two temperatures ($F=8.5$, $df=1;310$ $p<0.001$). The overall emergence percent at 4 and 8 °C were 26.2 ± 2.2 and 35.3 ± 2.2 , respectively. In the case of *T. achaeae*, the levels of parasitism significantly varied with storage time ($F=178$, $df=12;143$ $p<0.001$) at 4 °C. Its mean egg parasitism percent varied from 75 ± 2.8 at 0 week (no storage) to 2.8 ± 1.0 at the 8th week and no parasitism of eggs was observed after stored for 9 weeks or longer (Fig. 4). At 8 °C, levels of parasitism of *T. achaeae* significantly varied with storage time ($F=163.8$, $df=12;143$ $p<0.001$). Its mean egg parasitism percent ranged from 75 ± 2.8 at 0 week (no storage) to 6.3 ± 1.2 at the 10th week, while no parasitism of eggs was observed at storage for 11 weeks or longer (Fig. 4). It appears that parasitized host eggs can be stored over three weeks at 4 °C or four weeks at 8 °C and used for mass production, and further storage may not be economical as the level of parasitism drops down below 50%.

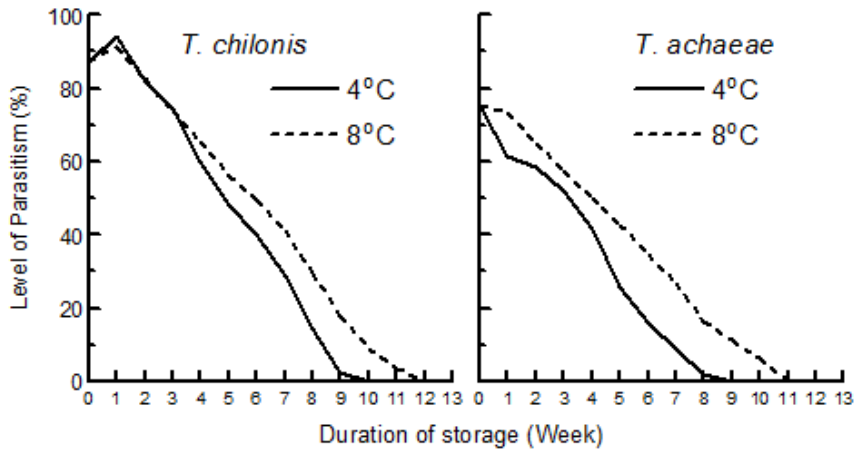


Fig. 4. Level of parasitism of emerged adults of *T. chilonis* and *T. achaeae* from cold stored parasitized eggs in different temperatures at 4 and 8 °C.

With respect to *T. chilonis*, level of parasitism did not vary significantly ($p > 0.05$) with temperature. The overall emergence per cent was 43.7 ± 1.9 . However, its levels of parasitism significantly varied with storage time ($F=455$, $df=12;299$ $p < 0.001$). The mean egg parasitism per cent varied from 87.1 ± 1.8 at 0 week (no storage) to 1.7 ± 0.8 at the 11th week. No parasitism was observed eggs stored for 12 weeks or longer (Fig. 4). It appears that parasitized host eggs can be stored over 5 weeks and used for mass production, and further storage may not be economical as the level of parasitism drops down below 50%.

(c) Assessment of suitable food for adult *T. chilonis*

Source of food

Longevity of *T. chilonis* was significantly different when they were fed on different food types ($F=162.1$, $df=5;594$ $p < 0.001$). Parasitoids lived longer period of time when they were fed on bee honey than glucose, sucrose, fructose and distilled water (Fig. 5). The highest longevity was recorded (17.87 ± 0.5 days) on undiluted bee honey. The shortest longevity was recorded on distilled water (2.64 ± 0.5 days). Longevity of parasitoids that were maintained without any food was marginally higher than those kept on distilled water. Similar results were recorded in a study conducted by Saljoqi and Khajjak (2007) with different parasitoid species.

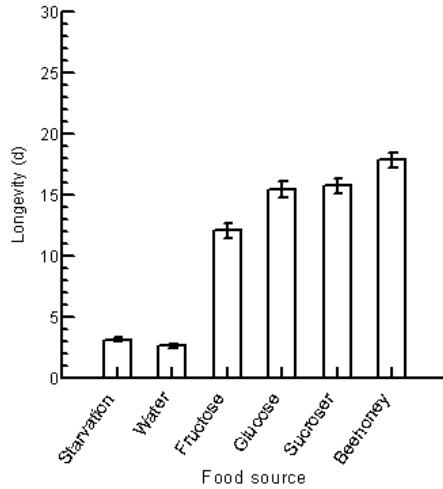


Fig. 5. Variation of adult lifespan of *T. chilonis* when adults were fed with different sources of sugar using a drop method in a clear plastic vial under laboratory conditions.

Suitable concentration of bee honey

Longevity of *T. chilonis* significantly varied with different concentrations of bee honey ($F=122.65$, $df=6;693$ $P<0.001$) (Fig. 6). The highest longevity 18.75 ± 0.5 days was found when they were fed with 50% bee honey. These results are similar with the findings of Saljoqi and Khajjak (2007) while the lowest longevity (3.77 ± 0.5 days) was recorded when the parasitoids were fed with distilled water.

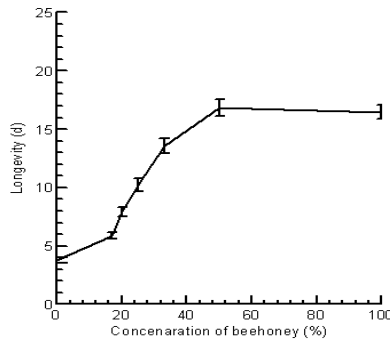


Fig. 6. Adult longevity of *T. chilonis* when they were fed on different concentrations of bee honey within a clear plastic vial under laboratory conditions.

Evaluation of different feeding methods for adult parasitoids

Feeding method may have an effect on safety of feeding for parasitoids and food intake that also could be measured by longevity. Inappropriate feeding methods led for accidental death of adult parasitoids due to sticking or submerging in the food source. Longevity of *T. chilonis* was significantly varied with feeding method ($F=86.596$, $df=3;396$ $p<0.001$).

The highest longevity was recorded in filter paper method 20.71 ± 0.5 days and the lowest longevity was recorded in wick method, 9.66 ± 0.5 days (Fig. 7). However practical usage of filter paper method is difficult in terms of labour and time consumption. Therefore, it was

suggested to use drop method over the filter paper method as a feeding method for parasitoids. Drop method, and sponge method showed 16.53 ± 0.5 and 10.14 ± 0.5 days longevity, respectively.

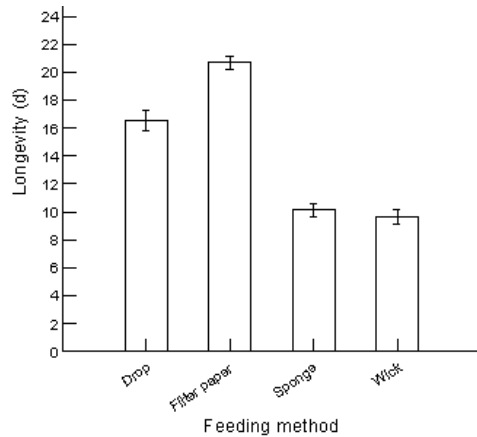


Fig 7. Adult longevity of *T. chilonis* when they were fed with bee honey on different feeding methods within a clear plastic vial under laboratory conditions.

(d) Potential for continuous rearing of *T. chilonis* and *T. achaeae* on *C. cephalonica* eggs

Level of parasitism significantly varied between species ($F=391$, $df=1;380$ $p<0.001$) and among generations ($F=71$, $df=9;380$ $p<0.001$). In addition, species x generation interaction was also significant ($F=3.3$, $df=9;380$ $p<0.001$). With respect to *T. chilonis*, capacity for parasitization of host eggs significantly varied with the generation ($F=28.8$, $df=9;190$ $p<0.001$). It was $84 \pm 2.9\%$ in the first generation and 31.6 ± 3.9 in the 10th generation (Fig. 8). However, the drop of parasitization capacity up to 5th generation was not significant ($p>0.05$). Similarly, with respect to *T. achaeae*, capacity for parasitization of host eggs significantly varied with the generations ($F=52.3$, $df=9;190$ $p<0.001$). It was 71.2 ± 3.5 in the first generation and 12.1 ± 1.7 in the 10th generation. The *T. achaeae* showed a steady decrease of capacity for parasitization compared with *T. chilonis* (Fig. 8). As the data indicated, *T. chilonis* could be mass cultured for relatively longer period on *C. cephalonica* eggs than *T. achaeae*.

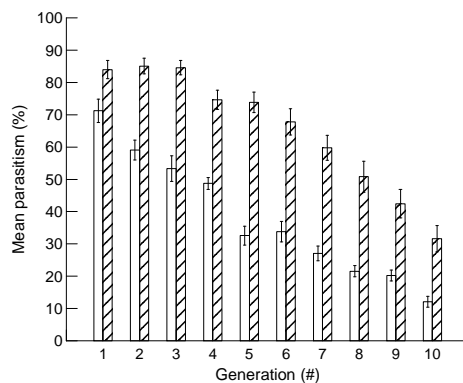


Fig. 8. Capacity of *T. chilonis* and *T. achaeae* for parasitization of host eggs with generations when the adults were continuously reared on *C. cephalonica* eggs under laboratory conditions. Open bar=*T. achaeae*.

CONCLUSIONS

Rearing *Trichogramma chilonis* and *T. achaeae* using *C. cephalonica* eggs as alternate host is technically feasible. Potential for using cool-stored eggs for parasitization was evident. The *T. achaeae* and *T. chilonis* respond to the stored eggs similarly. The level of acceptance of cool-stored *C. cephalonica* eggs for parasitization by both species declined with stored period. The *C. cephalonica* eggs, stored up to 2 weeks at 8 °C and 4 °C can be used for parasitoid rearing. *C. cephalonica* eggs containing pupal stage of the parasitoids, *T. chilonis* and *T. achaeae* can be stored alive up to three weeks at 4 °C and up to four weeks at 8 °C. Adult parasitoids can be successfully kept in the culture providing them with 50% diluted bee honey as a drop method. Rapid loss of parasitization capacity of *T. achaeae* was evident compared with *T. chilonis*.

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