Article

Role of temperature and hosts (*Sitotroga cereallela* and *Corcyra cephalonica*) egg age on the quality production of *Trichogramma chilonis*

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Abstract

The present study was conducted for efficient and quality production of the stingless wasp, *Trichogramma chilonis* Ishii with respect to rearing temperature and host egg age of the angoumois grain moth, *Sitotroga cereallela* (Olivier) and the rice meal moth, *Corcyra cephalonica* (Stainton) on its biology. Maximum parasitism was observed 95.7 and 84.3% at 28 °C, while minimum parasitism was 61.3 and 39.6% at 32 °C on *S. cereallela* and *C. cephalonica* eggs, respectively. The most favorable temperature was 28 ºC on which maximum parasitism and adult emergence were obtained from *S. cereallela* eggs. Maximum parasitism was observed 97.4 and 79.4% in 2 h old, while minimum parasitism was 24.6 and 17.3% in 72 h old eggs of *S. cereallela* and *C. cephalonica* eggs, respectively. Parasitism by *T. chilonis* decreased with increasing host eggs age. Maximum adult *T. chilonis* emergence was 98.2% in 2 h old eggs, while minimum emergence was 21.5% on 72 h old eggs of *S. cereallela*. Adult *T. chilonis* longevity on the host eggs of different ages of female wasp was non-significantly different to each other except the 2 and 12 h old eggs which were significantly different from rest of the treatments in both hosts' eggs of different ages. Maximum female longevity was 4.0 d on 2 h fresh eggs *C. cephalonica*, while minimum was 3.0 d on 24–48 h old *S. cereallela* eggs. The female ratio for different host eggs age was almost non-significant to each other except 2 h old eggs with maximum number of female (64). The results showed that *T. chilonis* preferred young eggs when offered older eggs, simultaneously.

Keywords adult longevity; *Corcyra cephalonica*; developmental period; female ratio; host age; parasitism; *Sitotroga cerealella*; temperature; *Trichogramma chilonis.*

1 Introduction

Biological control is relatively permanent, safe, economic and environmental friendly (Debach, 1974). The crop management practices include protects and encourages natural enemies and increases their impact on pests for conservation as a biological control method. The selective use of natural enemies, which are planting by strip on crops in and around the fields, necessarily food and habitat are provided to them, have been exercised. The parasitoid stingless wasp, *Trichogramma chilonis* (Ishii) has been conserved along with other natural enemies in Pakistan (Irshad, 2008).

The mass rearing and quality production of *T. chilonis* depends on several factors, i.e., temperature,

relative humidity, photoperiod and host egg quality. For rearing of *T. chilonis* on a commercial scale, it is necessary to use a factitious rearing host, such as the Mediterranean flour moth, *Ephestia kuehniella* (Zell), the angoumois grain moth, *Sitotroga cereallela* (Olivier) and the rice meal moth, *Corcyra cephalonica* (Stainton) rather than the natural or target host. The choice of factitious host is often dictated by the ease of rearing and not necessarily by any factors related to the likely success of the wasps being produced. Factitious hosts are selected on the simplicity of their mass production, mechanization of rearing processes and cost of production compared with that of using the target pest (Greenberg et al., 1998). Environmental factors and host quality can effect developmental period, longevity, parasitism, adult emergence from parasitized eggs and sex ratio (Corrigan and Laing, 1994).

Egg parasitoid, *Trichogramma chilonis* is an important biological agent for the control of sugar cane borers, *Diatraea saccharalis* (Fabricius) (Ashraf and Fatima, 1993). *Sitotroga cerealella* originally proposed by Flanders (1930), is one of the most commonly used as fictitious host for rearing *Trichogramma sp*. It is known that host age is one of the most important factors determining host acceptance in insect parasitoids (Vinson, 1985). In a *T. chilonis* production facility, the host eggs are collected at specified time intervals. The age of host eggs involves in *T. chilonis* production in at least two ways. Firstly, the oviposition preference of the parasitoid females (Pak, 1986) and secondly, as an indicator of the resource quality available for the developing parasitoid larvae thus affecting the physiology of host parasitoid interaction (Vinson and Iwantsch, 1980). Guang and Oloo (1990) and Schmidt et al. (1999) found that *T. chilonis* significantly decreased its parasitization when the eggs were older than 48 h at the time of encounter. Farid et al. (2001) found that *T. chilonis* preferred young eggs when offered along with older eggs simultaneously. Old eggs were not parasitized in the presence of younger eggs. Under no choice test, the parasitoids offered only one age group host at a time, parasitism, adult emergence, adult longevity did not differ among different age groups of host. Only difference of host age is that the female ratio of emerging *T. chilonis* dropped below 1%. The present research was conducted to check the effect of temperature and host eggs age on biology, i.e., parasitism, adult emergence, adult longevity and sex ratio of *T. chilonis* feeding on *S. cereallela* and *C. cephalonica* eggs under laboratory conditions for efficient and quality production of *T. chilonis*.

2 Materials and Methods

2.1 Insects rearing

For the present research, the cultures of *S. cerealella* and *C. cephalonica* were maintained under laboratory condition on the natural diet, i.e., wheat grains. The rearing temperature was maintained at 28±1 ºC, 16 h light: 8 h dark photoperiod and 50-60% relative humidity in the Biological Control Laboratory (BCL), Insect Pest Management Programme (IPM), Institute of Plant and Environmental Protection (IPEP), National Agriculture Research Centre (NARC), Islamabad, Pakistan during July-December, 2010 (Henderson, 1993; Perveen and Sultan, 2012).

2.2 Angoumois grain moth, *Sitotroga cerealella*

For rearing, eggs of *S. cerealella* were released in trays (36×30×5 cm) having 5 kg wheat grains in upper part of mass rearing chamber made up of tin sheets, consisted of two parts, its upper part was rectangular (37×37×50 cm) while lower was tapered downward with an opening (3×3 cm). Adults collecting box was attached with it. Upon hatching larvae were fed on wheat grains, then pupated and adults were emerged within 25–30 days and dropped directly into adults collecting box. When it contained sufficient number of adults, was daily replaced with empty one. Adults were placed on starch in plastic plates, they laid eggs, were collected by

sieving starch with 80 no of mesh sieve. The eggs were used for further experimental purpose (Perveen and Sultan, 2012).

2.3 Rice meal moth, *Corcyra cephalonica*

Under the same laboratory conditions, for maintenance of culture of *C. cephalonica*, 150-200 eggs were mixed daily up to 25 days in the jars containing 1½ kg of rice grain in transparent glass jars (22×55 cm), then the jars were covered with muslin cloth. The larvae fed on rice grain and moulted then pupated. Adults were emerged after 60–75 days. The adult female laid eggs inside of the muslin cloth and then eggs were collected with brush and used for further experimental purpose (Perveen and Sultan, 2012).

2.4 Parasitoid: Stingless wasp, *Trichogramma chilonis*

The culture of *T. chilonis* was maintained under the same laboratory conditions on eggs of *S. cerealella and C. cephalonica* were glued on hard paper (8×3 cm²) and put in glass jars (55×12 cm) having *T. chilonis* for parasitization. Adults *T. chilonis* usually emerged after 8-9 days and were provide with a new fresh cards for culture maintenance continuously (Perveen and Sultan, 2012).

2.5 Identification of sex of parasitoid

Parasitized eggs were kept in transparent gelatin capsule for adult emergence. Then under a high power stereoscope, parasitoid, *T. chilonis* male with long antennae and whorl of hairs and small segments, while female with smaller antennae with tiny hairs and large terminal segments have been identified (Perveen and Sultan, 2012).

2.6 Effect of temperatures and host eggs age on biology of parasitoids

Effects of three different temperatures, 24, 28 and 32 ºC and eggs of different ages (2, 12, 24, 48, 72 h) of *S. cerealella* and *C. cephalonica* was studied on the biology of *T. chilonis* in a growth chamber.

For no choice test, 100 eggs of *S. cerealella* and *C. cephalonica* of different ages (2, 12, 24, 48, 72 h) (24 h for different temperature) were glued per white paper card (8×3 cm²), separately. Each egg card (one for age or one for temperature, separately) was placed singly in glass jar [3.5 cm (dm)×12 cm (l)] contained 30 pairs of one day old male and female *T. chilonis*. The cards were removed after 24 h and placed at 28±1 ºC (for host eggs age); 24, 28, 32 ºC (for three different temperatures) and 50-60% RH to allow the development of *T. chilonis*. The experiment was arranged completely randomized with 10 replications for each respective egg age/ temperature. During experiment, adult *T. chilonis* were fed 10% honey solution for all treatments. Data of the following biology of adults *T. chilonis* were recorded: parasitism (calculated by counting the eggs that turn black), adult emergence from parasitized eggs, female ratio, developmental period and adult longevity.

2.7 Data analysis

Data were analyzed by one-way analysis of variance (ANOVA) (Concepts, 1989) at $P<0.05$ using LSD test.

3 Results

3.1 Effect of temperature on parasitoids

The results indicate that on *S. cereallela* eggs, *T. chilonis* parasitism was not significantly different at 24 and 28 °C, while it was significantly different at 32 °C. However, adults' emergence, female ratios, developmental period and adults' longevity were significantly different at three tested temperatures (Table 1).

On *C. cephalonica* eggs, *T. chilonis* parasitism, adults' emergence and female ratios were significantly different at three tested temperatures. However, developmental period and adults' longevity were not significantly different at 28 and 32 °C, while they were significantly different at 24 °C (Table 2).

Table 1 Effect of rearing temperatures on biology of *Trichogramma chilonis* on *Sitotroga cereallela* eggs*

Treatments temperature °C	n	Parasitism (M±SD)	Adult emergence (M±SD)	Female ratio (M±SD)	Developmental period (d) (M±SD)	Adult longevity (d) (M±SD)
24	10	92.30±4.92[a]	89.40±5.33[b]	51.0±2.39[b]	9.6±0.32[a]	4.3±0.38[a]
28	10	95.70±1.94[a]	96.30±3.45[a]	59.2±5.83[a]	8.2±1.08[b]	3.6±0.41[b]
32	10	61.30±1.70[b]	51.10±1.41[c]	58.1±4.13[c]	7.4±0.36[c]	2.0±0.56[c]

*Data were analyzed by using one-way analysis of variance (ANOVA) (Concepts, 1989) at $P<0.05$. Means within columns followed by different letters are significantly different by LSD test; M±SD: mean±standard deviation; d: days.

Table 2 Effect of rearing temperatures on biology of *Trichogramma chilonis* on *Corcyra cephalonica* eggs*

Treatments temperature °C	n	Parasitism (M±SD)*	Adult emergence (M±SD)	Female ratio (M±SD)	Developmental period (d) (M±SD)	Adult longevity (d) (M±SD)
24	10	74.2±7.33[b]	71.2±7.26[b]	57.6±5.46[b]	9.2±5.46[a]	4.1±0.31[a]
28	10	84.3±2.26[a]	80.3±4.85[a]	58.3±12.03[a]	8.0±0.64[b]	4.0±0.30[a]
32	10	39.6±4.57[c]	34.8±3.55[c]	54.3±0.97[c]	7.2±0.36[b]	2.6±0.44[b]

*Data were analyzed by using one-way analysis of variance (ANOVA) (Concepts, 1989) at $P<0.05$. Means within columns followed by different letters are significantly different by LSD test; M±SD: mean±standard deviation; d: days

Table 3 Effect of host eggs age on the biology of *Trichogramma chilonis* on *Sitotroga cereallela* eggs*

Treatments h old	n	Parasitism (M±SD)	Adult emergence (M±SD)	Female ratio (M±SD)	Adult longevity (d) (M±SD)
2	10	97.40±0.84[a]	98.20±0.94[a]	64.2±1.64[a]	3.8±0.27[b]
12	10	96.30±0.67[a]	91.30±4.52[b]	58.2±3.32[b]	3.9±0.59[a]
24	10	79.40±4.47[b]	76.50±1.13[c]	58.1±3.36[c]	3.0±0.32[c]
48	10	59.4±5.18[c]	52.3±1.28[d]	58.2±2.51[d]	3.0±0.32[c]
72	10	24.6±4.92[d]	21.5±1.33[e]	58.4±1.19[e]	3.1±0.25[c]

*Data were analyzed by using one-way analysis of variance (ANOVA) (Concepts, 1989) at $P<0.05$. Means within columns followed by different letters are significantly different by LSD test; M±SD: mean±standard deviation; d: days

3.2 Effect of host eggs age on parasitoids

The results indicate that *T. chilonis* adults' emergence and female ratios were significantly different at tested age eggs groups of *S. cereallela*. However, parasitism was not significantly different at 2 and 12 h old eggs of *S. cerealella*, moreover, it was significantly different at 24, 48 and 72 h old eggs of host. Further, adult longevity was significantly different at 2 and 12 h old eggs, furthermore, it was not significantly different at 24, 48 and 72 h old eggs of host (Table 3).

Trichogramma chilonis were fed on *C. cephalonica* eggs the same ages as mentioned for *S. cerealella*. The results indicate that *T. chilonis* parasitism, adults' emergence and female ratios were significantly different at 24, 48 and 72 h old eggs of *C. cephalonica*. However, they were not significantly different at 2 and 12 h old eggs. Moreover, adult longevity was significantly different at 2 h old eggs of host. Further, it was not significantly different at 12, 24, 48 and 72 h old eggs h old eggs (Table 4).

Table 4 Effect of host eggs age on the biology of *Trichogramma chilonis* on *Corcyra cephalonica* eggs*

Treatments h old	n	Parasitism (M±SD)	Adult emergence (M±SD)	Female ratio (M±SD)	Adult longevity (d) (M±SD)
2	10	79.4±4.47^{a}	69.4±3.98^{a}	55.5±1.42^{a}	4.0±0.30^{a}
12	10	78.3±3.56^{a}	71.0±3.56^{a}	56.1±1.22^{a}	3.3±0.21^{b}
24	10	64.6±4.71^{b}	62.2±4.58^{b}	58.2±2.98^{b}	3.1±0.43^{b}
48	10	41.2±2.69^{c}	61.6±2.36^{c}	61.8±2.51^{c}	3.1±0.35^{b}
72	10	17.3±2.83^{d}	22.5±0.73^{d}	61.5±0.51^{d}	3.2±0.53^{b}

*Data were analyzed by using one-way analysis of variance (ANOVA) (Concepts, 1989) at $P<0.05$. Means within columns followed by different letters are significantly different by LSD test; M±SD: mean±standard deviation; d: days

4 Discussion

On *S. cereallela* eggs, maximum parasitism 95.7% was observed at 28 °C followed by 92.3% at 24 °C, while minimum parasitism was 61.3% at 32 °C. The parasitism was non-significant to each other at 24 and 28 °C, while, it was significantly at 32 °C. The results further 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 84.3% at 28 °C followed by 74.2% at 24 °C and minimum parasitism was 39.6% at 32 °C. The results were significantly different from each other at three different temperatures. They further indicate that among two host eggs, *S. cereallela* were significantly different from *C. cephalonica* eggs for parasitism. Greater parasitism was obtained from *S. cereallela* as compared to *C. cephalonica* eggs at all temperatures. The most favorable temperature was 28 °C. Further, results indicate that parasitism on both host eggs were significant to each other at same temperature. The effects of temperature on *T. chilonis* were also at par with the most of earlier studies. Some strains of *T. chilonis* could tolerate up to 37±1 °C (Singh et al., 2002). Calvin et al. (1984) introduced 30 °C as the optimum temperature for *T. chilonis* development. However, Miura and Kobayashi (1993) demonstrated that 28 °C was the optimum temperature for *T. chilonis* developing on eggs of diamond back moth which were in agreement with the present study that 28 °C was the optimum temperature for *T. chilonis* on eggs of *S. cereallela* which was significantly different from *C. cephalonica* eggs at the same temperature.

In the present studies, maximum adult *T. chilonis* emergence from *S. cereallela* parasitized eggs was 96.3% at 28 °C followed by 89.4% at 24 °C and 51.1% at 32 °C which indicates that adult emergence were significantly different at three temperatures. Similarly, adult *T. chilonis* emergence on *C. cephalonica* eggs were 80.3% at 28 °C followed by 57.6% at 24 °C and 54.1% at 32 °C. The results further indicate that among two host eggs, *S. cereallela* were significantly different from *C. cephalonica* eggs for adult *T. chilonis* emergence at 28 and 24 °C. Greater adult *T. chilonis* emergence was obtained from *S. cereallela* eggs as compared to *C. cephalonica* eggs at initial two temperatures. The most favorable temperature was 28 °C.

To check the effects of host insect eggs age, i.e., 2, 12, 24, 48 and 72 h old eggs of *S. cereallela.* The results indicate, parasitism by *T. chilonis* were 97.4, 96.3, 79.4, 59.4 and 24.6%, respectively. The present study indicates that 2 and 12 h old eggs of *S. cereallela* were non-significant to each other but significantly different than the rest of the treatments for parasitism by *T. chilonis*. Maximum parasitism 97.4% was in 2 h while minimum parasitism 24.6% was in 72 h old eggs. The results further indicate that parasitism by *T. chilonis* decreased with increasing host eggs age. The results indicate that significant decrease with different treatments except the first two as in *S. cereallela* eggs. The result further indicates that both host eggs of different ages were significantly different for parasitism by *T. chilonis.* The present results confirmed the results of Schmidt et al. (1999) who found that *T. chilonis* significantly decreased parasitism when the eggs were older than 48 h at the time of encounter. The host eggs ages at time of parasitism appears to have implication on the fitness of progeny and parasitoids which preferentially attack younger host eggs (Sequeira et al., 1988, Renzik et al., 1997).

The present study further indicates that maximum adult *T. chilonis* emergence was 98.2% in 2 h old eggs of *S. cereallela,* while minimum emergence was 21.5% in 72 h old eggs. The results of this aspect were significantly different except the first two, 2 h and 12 h old eggs.

The study further indicates the female ratio for different host eggs age were almost non-significant to each other except the 2 h old eggs, with maximum number of female (64.2%) on *S. cereallela* eggs. Adult *T. chilonis* longevity of female wasp was non-significantly different to each other except the 2 and 12 h old eggs which were significantly different from rest of the treatments in both insects' eggs of different ages. Maximum female longevity was 3.9 d on 12 h fresh eggs while minimum was 3.0 d on 24 h and 48 h old *S. cereallela* eggs. Further research should be needed to clarify this mechanism.

5 Conclusion

On the basis of present study it is concluded that *S. cereallela* eggs more suitable as compare to *C. cephalonica* eggs for mass and quality rearing of parasitoid, *T. chilonis* at most favorable temperature 28°C when provide fresh eggs of 2-12 h old.

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References

Ashraf M, Fatima B. 1993. Control of sugarcane borers by inundative releases of *Trichogramma chilonis* (Ishii). Pakistan Journal of Zoology, 25: 23-25

Calvin DD, Knapp MC, Welch SM, et al. 1984. Impact of environmental factors on *Trichogramma pretoisum*

reared on southwestern corn borer eggs. Environmental Entomology, 13: 774-780

Concepts A. 1989. Super ANOVA. Abacus Concepts, Berkeley, CA, USA

Corrigan JE, Laing JE. 1994. Effects of the rearing host species and the host species attacked on performance by *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae). Environmental Entomology, 23: 755-760

Debach P. 1974. Biological Control by Natural Enemies. Cambridge University Press, London, UK

Farid A, Saqib T, Khan AU, et al. 2001. Host age effect on oviposition preference and development of Trichogramma chilonis. Pakistan Journal of Biological Sciences, 4(2): 121-122

Flanders SE. 1930. Mass production of egg parasites of genus *Trichogramma*. Hilgardia, 4: 465-501

Greenberg SM, Morrison RK, Nordlund DA, King EG. 1998. A review of the scientific literature and methods for production of factitious hosts for use in mass rearing of *Trichogramma spp*. (Hymenoptera: Trichogrammatidae) in the former Soviet Union, the United States, Western Europe and China. Journal Entomology Science, 33: 15-32

Guang LQ, Oloo GW. 1990. Host preference studies on *Trichogramma sp. nr*. *Mwanzai* Schulten and Feinjen (Hymenoptera: Trichogrammatidae) in Kenya. Insects Science Application, 11: 757-763

Henderson K. 1993. Effect of host and parasite density on the egg parasite Trichogramma pretiosum (Hym., Trichogrammatidae). Entomophaga, 26: 445-451

Irshad M. 2008. Biological control of insects and weeds in Pakistan. H. E. C. Isb., 1-315

Miura K, Kobayashi M. 1993. Effect of temperature on the development of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), an egg parasitoid of the diamondback moth. Journal Applied Entomology, 28: 393-396

Pak GA. 1986. Behavioral variation among strains of *Trichogramma spp*: A review of the literature on host selection. Journal Applied Entomology, 101: 55-64

Perveen F, Sultan R. 2012. Effects of the host and parasitoid densities on the quality production of *Trichogramma chilonis*on lepidopterous (*Sitotroga cereallela* and *Corcyra cephalonica*) eggs. Arthropods, 1(2): 63-72

Reznik SY, Umarova TY, Voinovich ND. 1997. The influence of previous host age on current host acceptance in *Trichogramma*. Entomologia Experimentalis et Applicata, 82(2): 153-157

Schmidt M, Mandel G, Schmuck R. 1999. Impact of *Vairimorpha sp.* (Microsporidia: Burnellidae) on *T. chilonis* (Ishii) a hymenopteran parasitoid of the cabbage moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). Journal Invertebrate Pathology, 74: 120-126

Sequeira R, Mackauer M. 1988. Effects of parasitism by *Praon pequdorum* on age specific fecundity and population growth of the pea aphid, *Acyrthosiphon pisum*. Entomologia Experimentalis et Applicata, 48: 179-185

Singh SP, Singh J, Brar KS. 2002. Effect of temperature on different strains of *Trichogramma chilonis* Ishii. Insect Environment, 7: 181-182

Vinson SB, Iwantsch GF. 1980. Host regulation by insect parasitoids. Quaternary Review of Biology, 55:143-165

Vinson SB. 1985. The behavior of parasitoids. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology (Kerkut GA, Gilbert LI, eds). 417-469, Pergamon Press, Oxford, UK