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Studies on the biological attributes and parasitic potential of *Trichogramma Chilonis* (Ishii) reared on *Sitotroga cerealella* (Olivier) and *Corcyra cephalonica* (Stainton) eggs under laboratory conditions

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Abstract
Research experiments were conducted to check the effect of two fictitious hosts, *Sitotroga cerealella* and *Corcyra cephalonica* eggs on biological attributes of *Trichogramma chilonis*at Insect pest management program, Bio control laboratories, National Agriculture Research Centre, Islamabad. Both free choice and no choice techniques were exercised. Maximum percent parasitism (94.0%) was observed on *S. cerealella* and (88.0%) on *C. Cephalonic a*eggsunder no choice method. Host's eggs age study indicates that *T. Chilonis* preferred (12 to 24) hours old eggs of both host insects. Host eggs age drastically reduced emergence of adults as the age of the eggs proceed. A single female *T. Chilonis* parasitized (26.4) and (25.0)eggs of both S. cerealella and *C. Cephalonic a*respectively. Maximum emergence (26.1%) by *T. Chilonis* was observed in *S. Cerealella* when the parasitoid was reared on 30 eggs/density. The results of the present studies indicate that *T. chilonis* can successfully be mass reared on eggs of both host insects. On the basis of the present results *S. cerealella* eggs was more suitable for mass production of *T. Chilonis*. However, *C. cephalonica* eggs may also be used as an alternate host for mass production of *Trichogramma* under controlled conditions.

Keywords: *Trichogramma chilonis*, *Sitotroga cerealella* and *Corcyra cephalonica* eggs, biological attributes, Parasitism, adult emergence

1. Introduction
The ecological application of the genus *Trichogramma* has been studied intensively because of their importance as biological control agents for use in a variety of agro ecosystems, such as corn, sugarcane, rice, soybean, cotton, sugar beet, vegetables and pine trees [11]. *Trichogramma* has several attributes including short life cycle and high reproductive potential that make them potentially useful as biological control agents and contribute to produce a rapid population increase [9]. In the 1960s, the Europeans and Americans revitalized research on *Trichogramma* and in the 1970s, they began mass rearing and releases [15].

T. chilonis(Hymenoptera: *Trichogrammatidae*) is commonly distributed group in the world including Pakistan. It has been reported from different location in Pakistan containing Rawalpindi, Peshawar, Matli, Larkana and Lahore [8]. [1] stated that the interest in these parasitoids (*Trichogramma* spp.) as bio control agent is evident because of killing the pest at the most critical stage (the egg) prior to occurrence of damage. It was reported that release of *Trichogramma* spp. reduced the infestation level of sugar cane stem borer [13].

Trichogramma spp. are easily reared on fictitious hosts i.e. *Sitotroga cerealella* and *Corcyra cephalonica* eggs under controlled conditions [6]. In Pakistan *Trichogramma* are usually reared by public and private sector on *S. cerealella* eggs. But *C. cephalonica* eggs can also be used as an alternate host for mass production of *Trichogramma.* [3]reported 93.86% parasitism on *C. Cephalonia* egg. *C. cephalonica* is used as a host of more than 60 natural enemies of which 15 are parasitoids. Suitability to wide range and its unique character is beneficial for the production of biological control agents on large scale. At a number of commercial insectaries and industries in South Asia, *C. cephalonica*ica reared on large scale to serve as egg parasitoid host for many bio control agents [7].

The present studies were intended to find out most suitable host out of two fictitious lepidopterous hosts, *S. cerealella* and *C. cephalonica* eggs for quality mass production of *Trichogramma* keeping in view high percent parasitism, adult emergence host's eggs age influence and also the influence of host's eggs density on the performance of the vary parasitoid insect.

2. Material and methods
Study was carried out to identify the impact of *Sitotroga cerealella* and *Corcyra cephalonica* on biological attributes of *Trichogramma chilonis* under laboratory conditions of

26±1ºC and 60-70% relative humidity with photo-period is 24 hours in Insectary at Insect Pest Management Program (IPMP), National Agricultural Research Centre (NARC) Islamabad during 2017.

2.1 Biological parameters of *Trichogramma chilonis* reared on *Sitrotergaa Cerealella* and *Corcyra cephalonica* eggs under free choice and no choice methods

A total of 100 eggs of *C. cephalonica* and *S. cerealella* under no choice method while a total of 200 eggs of *S. cerealella* and *C. cephalonica* each with 100 eggs on the same card were stuck with glue and placed in transparent glass jars having 50 parasitoids eggs for 24 hours in case of free choice. The experiments were replicated 10 times. Data were recorded on parasitized and un-parasitized eggs, adult emergence, developmental period and adult parasitoid longevity on both host eggs.

2.2 Effect of host's egg age on biological parameters of *Trichogramma chilonis*

2.2.1 No choice and free choice method

100 eggs of different ages of *C. Cephalonica* and *S. cerealella*(12, 24, 48, 72 and 96 hours) were glued separately on card under no choice test. While a total of 200 eggs of different ages (each host insects with 100 eggs) were stuck on the same card under free choice method. Each egg card (same size) was put individually in 12 x 3 cm^2 glass jar; comprising 50 parasitoids. The cards were removed after 24 hours and percent parasitism was calculated by counting the eggs that turned black. The experiment was carried out in Completely Randomized Design (CRD). It was replicated 10 times and data were recorded on the following parameters.

a. Percent parasitism
b. Percent Adult emergence

2.3 Efficacy of host densities on the biological attributes of *T. chilonis*

The host insect's eggs (*S. cerealella* and *C. Cephalonica)* were fixed on the cards including densities of 10, 20, 30, 40 and 50 eggs each with 10 replicates. Prior to release of *Trichogramma* in transparent tubes consisting cards and pasted with different densities of both host eggs, the parasitoid' spairs were identified/isolated as male and female after emergence. For this purpose, a total of 200 single parasitized pupae were kept in transparent capsules singly. After emergence in capsule the individuals were observed under high powerful digital microscope and separated on the basis of their antennae where male possessing extended antennae having spiral hairs and slight segments and female has small antennae having small hairs and witn huge lethal segments.

After thoroughly identification a pair of *Trichogramma* was released in test tubes consist of different densities of both host insects eggs. Plastic sheet were used to cover the top of the test tubes and binded with the help of rubber strings. On next day the *Tricho*-cards consisted various densities of host eggs were removed and kept for checking parasitism and adult parasitiod emergence in each test tube at all prey densities.

2.4 Statistical Analysis

The data recorded after conducting the aforementioned studies were subjected to analysis of variance with one way ANOVA while the data on percent parasitism and parasitoid adults emergence under free choice methods were subjected to analysis of variance with two way ANOVA (Two factor factorial test) using using Statistical package (Statistix 8.1). Means were separated using LSD test at 5% level of probability [5].

3. Results

3.1 Effect of *S. cerealella* and *C. cephalonica* eggs on parasitism and adult parasitoid emergence, adult durations under free choice and no choice methods

3.1.1 Free choice method

The results pertaining in table 1 indicate that rate of parasitism was significantly different from each other and a maximum parasitism of 92.0 % ±0.37 and minimum of 84.0 % ±0.37 was recorded the *Trichogramma* was reared on *S. cerealella and C. cephalonica* eggs under free choice method, respectively. Similarly maximum adult emergence of parasitoid (88.0% ±0.2981) was observed from parasitized eggs of *S. cerealella* and minimum (78.0% ±0.4944) from eggs of *C. cephalonica*, respectively (Table 1). Adult emergence was also statistically significant from each other when reared on two host eggs. Significantly maximum duration of 9.40%±0.1633 days form parasitism up to parasitoid' s adult emergence as compared to 9.0%±0.2108 days was noticed when reared on *S. cerealella* and *C. cephalonica* eggs, respectively (Table 1). Similarly, total duration of *Trichogramma chilonis* adultwas noted to be maximum (3.4±0.2211) days and (3.0±0.2582) days, when reared on two different hosts eggs, respectively (Table 1).

3.1.2 No choice method

Studies conducted under no choice method indicate that maximum parasitism (94.0% ±0.3333) occurred in eggs of *S. cereallela* while significantly minimum (88%±0.7601) recorded in eggs of *C. Cephalonica*, respectively, when the parasitoid was reared on eggs of two different hosts, (Table 2). The adult *T. chilonis*e mergence was maximum (94.0%±0.4944%) and minimum (89.0%±0.3651) parasitizing two different hosts eggs, respectively. A maximum of 9.6±0.1633 days were spent since parasitism up to adult parasitoid emergence when reared on *S. Cereallela* egg sas compared to a minimum of 9.0±0.2582 days when reared on *C. cephalonica* eggs respectively. The duration on both hosts insect eggs were significantly different from each other. Adults of *T. chilonis* remained live up to significantly maximum (3.6±0.2667) and (3.0±0.2582) days, respectively, after emergence from the hosts eggs.

Overall results obtained under free choice and no choice methods pertained that eggs of *S. cerealella* were highly preferred by *T. chilonis* for parasitism and parasitoid adult emergence (Table 1 and 2).

Table 1: Biological parameters of *Trichogramma chilonis* (Ishii) reared on *Sitotroga cerealella* (Olivier) and *Corcyra Cephalonia* (Stainton) eggs under free choice method

Parameter	Host insect		LSD
	***Sitotroga cerealella* eggs**	***Corcyra cephalonica* eggs**	
%parasitism	92.0 ± 0.37a	84.0 ± 0.37b	0.0283
%adult emergence	88.0 ± 0.30a	78.0 ± 0.49b	0.106
Total duration in days	9.40 ± 0.16a	9.0 ± 0.21b	0.0232
Adult duration	3.40 ± 0.22a	3.0 ± 0.26b	0.0242

Means followed by the same lower-case letter Row wise are non-significantly different at 5 ≤ 0.05 using LSD test.

Table 2: Biological parameters of *Trichogramma chilonis* (Ishii) reared on *Sitotroga cerealella* (Olivier) and *Corcyra cephalonia* (Stainton) eggs under no choice method

Parameter	Host insect		LSD
	***Sitotroga cerealella* eggs**	***Corcyra cephalonia* eggs**	
%parasitism	94.0± 0.33a	88±0.76b	0.0250
%adult duration	94.0± 0.49a	89± 0.37b	0.1525
Total duration in days	9.6± 0.16a	9.0± 0.26b	0.0284
Adult duration	3.6± 0.27a	3.0 ± 0.26b	0.172

Means followed by the same lower-case letter row wise are non-significantly different at 5 ≤ 0.05 using LSD test

3.2 Percent parasitism by *T. chilonis* and adult emergence from parasitized eggs under no choice method reared on *S. cerealella* eggs

The results in table 3 indicate that different ages of host insect eggs have significant effect on percent parasitism and adult emergence. The statistically highest percent parasitism (95±7.02) was noted in 24 hour old eggs followed by 12 hour (92.4±8.38). The parasitism at all tested host eggs age was significantly different from each other. The lowest percent parasitism (42±0.02) in 96 hour old eggs followed by 72 hours (60.4±0.02) and 48 hours of old eggs (82.4±0.01). A maximum of (91.3±8.91) percent adult's emerged in host eggs of 12 hours old followed by 24 hours (89±.01). The results also indicate that adults emergence from parasitized eggs were significantly different at all tested ages of eggs of both host insects. The percent adults emergence in 48 hours of host age was 69.4%±.01 followed by 72 hours old eggs (41.3%±.02) and the lowest percent adult emergence (13%±.01) were recorded in 96 hours old eggs (Table 3). The results of the present investigation indicates that 24 hours and 12 hours old eggs of both *S. cerealella* and *C. cephalonia* were most suitable host age for parasitism and parasitoid adult emergence. It was further indicated that as the host age increased the rate of parasitism and parasitoid adult emergence were significantly decreased.

3.3 Percent parasitism by *T. chilonis* and parasitoid adult emergence from parasitized eggs under no choice method reared on *C. cephalonica* eggs No choice method

Under free choice method the results revealed a significant difference between percent parasitism and percent parasitoid adult emergence at different ages of host insect eggs. Significantly highest percent parasitism was noted in 12 hours old eggs (91.3% ±.02) followed by 24 h old eggs (89%±0.01) and 48 h (69.4%±0.01). The lowest percent emergence was recorded in 96 h (13%±0.01) followed by 72 h (41.3%±0.0156). The percent emergence of adult parasitoids pertained that the highest number of adults emerged were (92.4%±8.384) in 12 h old eggs which was significantly different from the rest of host ages followed by 24 h (88%±0.0103). The percent adults' emergence in 48 hours old eggs were (56.3%±0.0157) followed by 72 hours (27.4%±0.0141) and no adult emergence was observed in 96 h old eggs.

Table 3: Effect of host ages on % parasitism and parasitoid adult emergence of *T. chilonis* reared on *S. cerealella* eggs under no choice method

Host age	*S. cerealella*	
	% Parasitism	**% Adult emergence**
12 hours	92.4±0.38b	91.3±0.91a
24 hours	95±0.02a	89±.01b
48 hours	82.4±0.01c	69.4±.01c
72 hours	60.4±0,02d	41.3±.02d
96 hours	42±0.02e	13±.01e
LSD	1.0331	0.8556

Means followed by the same lower-case letter Colum wise are non-significantly different at ≤ 0.05 using LSD test

Table 4: Effect of host ages on % parasitism and parasitoid adult emergence of *T. chilonis* reared on *C. cephaloni ca*eggs under no choice method

Host age	*C. cephalonica*	
	% Parasitism	**% Adult emergence**
12 hours	91.3±0.02a	92.4±0.08a
24 hours	89±0.01b	88±0.01b
48 hours	69.4±0.01c	56.3±0.02c
72 hours	41.3±0.02d	27.4±0.01d
96 hours	13±0.01e	0.00±0.0e
LSD	0.8764	0.0309

Means followed by the same lower-case letter Colum wise are non-significantly different at 5 ≤ 0.05 using LSD test

3.4 Effect of *S. cerealella* and *C. cephalonia* eggs on percent parasitism by *T. chilonis* under free choice method

The results revealed that *T. chilonis* parasitized maximum (100.00 ±0.00a %) in 24 hours old eggs of *S. cerealella* followed by 12 hours old eggs, which was non significantly different from each other on both host insect eggs but significantly different from all other tested host age in both insect eggs under free choice method. The minimum parasitism was on host eggs of 96 hours old age on both host insect eggs. The results further indicated that as the host age increase the number of parasitism decreased in both insect eggs. A significant difference was observed in the rate of parasitism between two hosts insect eggs in host age of 72- and 96-hours old eggs. (Table 5).

3.5 Effect of *S. cerealella* and *C. cephalonia* eggs on percent adult *Trichogramma* mergence under free choice method

No statistical significance regarding percent emergence of *T. chilonis* was recorded between the eggs of *S. cerealella* which were 12 and 24 hours old under free choice method. No adults emergence was recorded in 96 h old eggs. The results further indicated the maximum percent emergence of *T. chilonis* in *C. cephalonica* at host age of 12 hours was maximum (97.0%±0.03) followed by 24 hours (93.0%±0.03) whereas the lowest percent emergence of *T. chilonis* on *C. cephalonica* was (0.00%±0.00) at host age of 96 hours (Table 6).

Table 5: Percent parasitism by *T. chilonis* reared on *S. cerealella* and *C. cephalonica* eggs under free choice method.

Host age	Host		Mean
	S. cerealella	*C. cephalonica*	
	%parasitism ±SE	%parasitism ±SE	
12 hrs	98 ±0.00a	96.0±0.01a	0.980 a
24 hrs	100.0±0.01a	95.0±0.02a	0.965 a
48 hrs	76.0±0.04b	71.0±0.05b	0.735 b
72 hrs	54.0±0.05c	42.0±0.05d	0.480 c
96 hrs	10.0±0.03e	5.0±0.02e	0.075 d
LSD	0.676	0.618	

Means followed by the same lower-case letter row and Column wise are non-significantly different at ≤0.05 (Two way anova) using LSD test.

Table 6: Percent Emergence by *T. chilonis* reared on *S. cerealella* and *C. cephalonica* eggs under free choice method.

Host age	Host		Mean
	S. cerealella	*C. cephalonica*	
	% Parasitoid emergence ±SE	%Emergence ±SE	
12 hrs	93.0±0.01a	92.0±0.03a	0.955 a
24 hrs	98.0±0.02a	96.0±0.03a	0.950 a
48 hrs	78.0±0.04b	64.0±0.05c	0.710 b
72 hrs	51.0±0.05d	37.0±0.05e	0.440 c
96 hrs	0.00±0.00f	0.00±0.00f	0.000 d
LSD	0.648	0.574	

Means followed by the same lower case letter row and column wise are non-significantly different at ≤0.05 (Two way Anova) using LSD test

3.6 Effect of prey density on parasitism and adult *T. chilonis* emergence from parasitized eggs of *S. cerealella* and *C. cephalonica*

The parasitism per female *Trichogramma* on different prey densities revealed that number of parasitism increased with increasing prey density. Maximum (26.4±0.37), *S. cerealella* eggs were parasitized/female when they were offered 30 eggs per density. Similarly maximum (25.±0.34) eggs of *C. cephalonica* were parasitized when they were offered 30 eggs per density. The results indicated that further increase in prey density has no significant effect on parasitism by *T. chilonis* reared on both host eggs which indicated the upper straight-line level for *Trichogramma* was 30 eggs/ card after rearing on both host eggs. It was also indicated that parasitism per female *Trichogramma* increased with increasing prey density of both host insects eggs up to a certain level (Fig 1).

The results regarding adult parasitoid emergence from different densities of both *S. cerealella* and *C. cephalonica* eggs showed the same trend as for parasitism. Maximum parasitoidadults emergence were 26.1 ±0.34 from *S. cerealella* eggs at density of 30 eggs per card. While maximum adult parasitoid emergence from *Corcyra cephalonica* eggs were (23.90±0.48) at 30 eggs/density which was significantly different from the rest of densities. The figure indicated the same straight-line level for adult emergence on both host eggs which was 30 eggs per density and no significant effect on adult emergence was recorded with further increase in host eggs but contrasting to that increase in prey densities resulted in less adult emergence after straight line level (Fig 2).

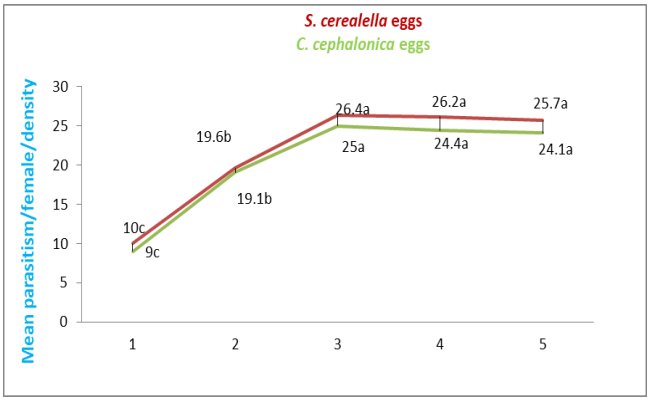

Fig 1: Numbers of *S. cerealella* and *C. cephalonica* eggs, parasitized/female *T. chilonis* at five constant densities

Means followed by the same lower case letter are non-significantly different at ≤ 0.05 using LSD test

Where 1= 10, 2=20, 3=30, 4=40 and 5=50 eggs per each density

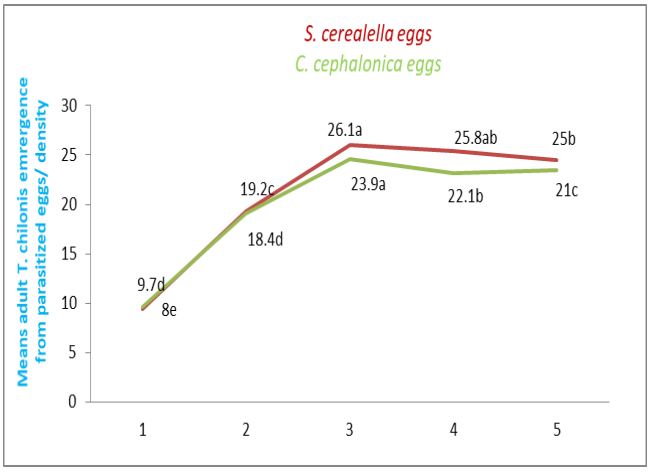

Fig 2: Numbers of adult *Trichogramma Chilonis* emerged from parasitized eggs of *S. cerealella* and *C. cephalonica* at five constant densities

Means followed by the same lower-case letter are non-significantly different at ≤ 0.05 using LSD test.

4. Discussion

Different biological attributes of *Trichogramma chilones* were studied on two fictitious hosts eggs of *S. cerealella* and *C. cephalonica* under both free choice and no choice methods. The results revealed significantly maximum parasitism (94.0% and 92.0%) on *S. cerealella* and minimum (88% and 91%) was observed on *C. cephalonica eggs* under no choice and free choice method, respectively. Similarly the results indicated that *Trichogramma* preferred

S. cerealella eggs for parasitism under both free choice and no choice method as compared to *C. cephalonica* eggs. Results of the present research are in conformity with the study of [10], who also reported that parasitism was maximum on *S. cerealella* eggs as compared to *C. cephalonia* eggs. Our study resulted in maximum adult parasitoid emergence when reared on *S. cerealella* eggs as compared to *C. cephalonica* eggs under bith free and no choice method. Results of [2] also indicated minimum parasitism and parasitoid adult emergence from parasitized eggs on *C. cephalonia* eggs as compared to *Dicladispa Armigera* eggs. Subsequently less preference of *Trichogramma* to *C. cephalonica* eggs for parasitism and parasitoid adult emergence is also obvious form the work of the previous researchers. The reason for their less preference for parasitism and adult parasitoid emergence may be due to different strain they used in their experiments. Regarding our research, less preference of the said parasitoidmay be due to slightly light weight and small round egg size eggs of *C. cephalonica* as compared to *S. cerealella* eggs, but still further investigation is needed.

The duration recorded from the parasitism up to adult parasitoid emergence was highest on *S. cerealella* eggs as compared to *C. cephalonica* eggs. The results of biological parameters under both free choice and no choice methods indicate significant differences among *S. cerealella* and *C. cephalonica* eggs. In the present study *S. ceraelella* eggs were proved more suitable as compared to *C. cephalonica* eggs on the basis of high parasitism rate, maximum parasitoid emergence and short durations. Our present findings are in coincidence with that of [10] who also reported *S. cerealella* eggs more suitable for *Trichogramma* mass rearing.

The results regarding host eggs age also indicated significant differences among different ages of host eggs under both free choice and no choice methods. Highest percent parasitism (100%) was noted in 24 h old eggs while lowest parasitism (10.0%) was recorded in 96 h old eggs. Similarly maximum parasitoid adult emergence were recorded in fresh eggs of 12 h and 24 h old eggs. The results further indicated that with increasing host eggs age, the rate of parasitism and parasitoid adult emergence were significantly decreased reared on both host insects eggs under both free choice and no choice method. [14]and[17] stated that parasitism decrease with an increase in host eggs age. [12]also reported that parasitoid preferred to attack the younger host eggs. Similarly [16] investigated maximum parasitism and parasitoid adult emergence in fresh eggs as compared to old eggs. Likewise, [4] studied in maximum parasitoid adult emergence from fresh eggs of host insects.

The present research study was also included to investigate the effect of five constant host densities of host insects eggs. The results revealed that parasitism per female *Trichogramma* tended to increase with increase in prey densities. The parasitism was maximum (26.40) eggs per density 30 eggs of *S. ceraelella*, while at the same density the maximum parasitism in *C. cephalonica* eggs were (25) per 30 eggs/density. The results further elaborated that prey densities behind 30 density cause no significant effect on parasitism by *Trichogramma* female. The same was also investigated by [10] who found maximum parasitism (85%) in *S. cerealella* and (68 %) in *C. cephalonica* eggs in 20 eggs/ densities.

5. Conclusion

It was concluded from the results of the present study that *Trichogramma chilonis* can successfully be mass reared on eggs of both host insects. Out of the two host insects' eggs, *S. cerealella* eggs were proved to be most suitable regarding parasitism, parasitiod adult emergence ratio and also due their short developmental duration. The most suitable age of eggs for parasitism was 12 to 24 hours fresh eggs. The most suitable host eggs densities were 30 eggs for single female *Trichogramma.*

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