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Effect of different temperatures on *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) parasitizing the eggs of *Corcyra cephalonica* and *Helicoverpa armigera*

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Abstract
The experiment was carried out to evaluate the temperatures requirements for the proper storage of trichocards parasitized by *Trichogramma chilonis* (Ishii) prepared from two host eggs i.e. *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). The effect of different temperatures treatment on the biological parameters like parasitization capacity, emergence percentage, adult longevity, sex ratio of *T. chilonis*, on the eggs of *C. cephalonica* and *H. armigera* were investigated. Parasitized trichocards were kept at 9^0, 11^0, 13^0, 15^0, 17^0, 19^0, 21^0, 23^0, 25^0 and 27 ^{0}C. Storage temperature of 15 ^{0}C showed the highest per cent parasitism (89.33% on *C. cephalonica* & 88.33% for *H. armigera* eggs) which was declining with increase in temperature, per cent adult emergence was 94 & 96% on *C. cephalonica* and *H. armigera* eggs respectively and total fecundity (99.33 & 103.67% on *C. cephalonica* and *H. armigera* eggs respectively) also followed the similar trend. Storage temperature of 9^0 recorded the highest longevity of *T. chilonis* (11.50 & 11.17 days, respectively on *C. cephalonica* and *H. armigera* eggs). Treatment with storage temperature of 13 ^{0}C recorded the highest sex ratio of female to male, 5:1 on *C. cephalonica* eggs and 4:1 on *H. armigera* eggs. In general the treatment with 15 ^{0}C was most effective overall recording the highest in three parameters and can be considered as the best treatment. The results suggested that the most suitable temperature for the storage of trichocards can be 13^0 to 15 ^{0}C.

Keywords: *Trichogramma*, temperature, storage, parasitization, emergence, longevity, sex ratio

Introduction
Parasitoids of the genus *Trichogramma* (Trichogrammatidae: Hymenoptera) are the most widely studied and successfully used parasitoids for biological control of many harmful lepidopteran insect pests of crops like sugarcane, paddy, cotton, corn etc. and vegetables [1, 2]. *Trichogramma* spp. are commonly distributed with over 200 species worldwide out of which India has about 26 species with *T. chilonis* as dominant one [3]. They parasitize the eggs of more than 400 lepidopteran pests [4]. 3 to 4 days after parasitization, dark melanin granules are deposited on the inner surface of chorion, turning it black and larva then pupate within the egg. After about 4-5 days, the adult wasp emerges from the pupae and escape from the host egg by chewing a circular hole in the egg shell. Some species enter a state of diapause which allows them to tolerate long periods of subfreezing temperatures. Trichogrammatids are minute, ranging from 0.2 to 1.5 mm in size [5].
Among the *Trichogramma* species the egg parasitoid, *Trichogramma chilonis* is the dominant species in India [6]. Integrated pest management by biocontrol agents needs the mass scale availability of biocontrol agents which requires factitious host capable of producing many generations successfully [7]. In India, Rice moth, *C. cephalonica*, a stored grain pest is being utilized in various biocontrol research, developmental and extension units for mass production of number of natural enemies [8]. It ranks first in the mass culturing of wide range of biological control agents due to its amenability to mass production, adaptability to varied rearing conditions and its positive influence on the progeny of the natural enemies.
Trichocards prepared from the different eggs are found suitable for the *Trichogramma chilonis* and its storage is an very valuable aspect in the quality control of the parasitoid which is relatively understudied with this view in mind current study was undertaken to understand the effective temperature/temperature range for the storage of trichocards prepared from the eggs

of *Corcyra cephalonica* and *Helicoverpa armigera*, former being the most commonly used factitious host for large number of egg parasitoids, and its effect on the different biological parameters of *T. chilonis*. Experiment was laid down in the biological control laboratory of department of entomology and agricultural zoology, institute of agricultural sciences, Banaras Hindu University, Varanasi,

Materials and methods:

The experiments were independently carried out for both host eggs (*C. cephalonica* and *H. armigera)* with a completely randomized experimental design with 10 different temperatures as a treatment and repeated 3 times.

Maintenance of culture

Rearing of *Corcyra cephalonica* (Stainton)

Culture of *C. cephalonica* was obtained from the IIVR, Varanasi and maintained in the controlled condition with 25 ± 1 °C temperature, 70 ± 10% relative humidity (RH), and 12h of light and 12h of darkness in the laboratory. The culture was then maintained on the fortified diet of maize. Maize grains free from any infestation were coarse grind and heat sterilized in hot air oven at 100 ^{0}C for 30 minutes. About 2.5 kg of the maize grains were mixed with 100 g of the groundnut powder and 5g powdered yeast was kept in plastic tray (45 x 30 x 10 cm). A spray of streptomycin sulphate 0.05 per cent was given @ 10-20 ml per tray to prevent bacterial infection. Sulphur 80 WP was added @ 5g per tray to prevent mite infection. *Corcyra* eggs 0.5cc (8000-9000 eggs) per tray was sprinkled uniformly in grain medium of each tray. This tray was covered with muslin cloth. The hatched larva feed on the grain by webbing. Full grown larva pupates inside the webbed grains mass for 5-7 days and adult moths emerged after 35-40 days from date of inoculation. The emerged *Corcyra* adults were collected daily and transferred in to a specially designed oviposition cage. Eggs were laid by the females were collected daily and used for making trichocards.

Rearing of *Helicoverpa armigera*

Helicoverpa larva were collected from the field and reared on the diet of chickpea seeds individually into the plastic vials till they pupate under controlled condition (at a temperature of 25 ± 1 °C, relative humidity (RH) of 70 ± 10%, and photoperiod of 12h of light and 12h of darkness) in the laboratory. After pupation the pupa were separated as male and female pupa and later on transferred into the jar for the adult emergence. In each jar one male and one female pupa were placed in order to facilitate the mating and oviposition. Once the eggs are laid in the oviposition chamber they were collected and used for further studies or maintaining the culture.

Culturing of *T. chilonis*

Nucleus culture of *T. chilonis* was procured from IIVR, Varanasi. Eggs obtained from both *C. cephalonica* and *H. armigera* were treated with UV rays (30 W UV tube for 45 minutes) to prevent hatching. The large egg cards (15 x 7.5 cm) were divided into 10 rectangular strips (3 x 2 cm) and thin layer of dilute Acacia gum was applied for pasting the eggs uniformly on the card @ 1cc per card. The card were kept in a large polythene bag (30 x 20 cm) containing nucleus egg card at the ratio of 1:6 to fresh eggs for parasitization by *T. chilonis* at room conditions with temperature of 26±2 ^{0}C and 65±5% RH. The emerging adults were used for further experiments. 50% honey solution will serve as adult food.

The parameters recorded were during the experiment were: 1) Percent parasitism; 2) Percent adult emergence; 3) Total fecundity/female; 4) Adult female longevity and 5) Sex ratio (F:M).

For each experiment three glass tubes (replications) were prepared, then they were kept inside the controlled environmental chambers at the temperatures of 9, 11, 13, 15, 17, 19, 21, 23, 25 and 27 ^{0}C ± 1 ^{0}C, 70 ± 10% RH, and 12 h of light and dark each.

Percent parasitism of the *T. chilonis* on *C. cephalonica* and *H. armigera*

The experiment was conducted under a Completely Randomized Design (CRD) with two different host eggs *H. armigera*, and *C. cephalonica*. Adults of *T. chilonis* were allowed to parasitize the eggs strips prepared earlier for 24 h, on 4th day parasitized eggs turned black in colour. At this stage egg cards strip were kept in separate test tubes for adult emergence. These egg strips were replaced daily until the death of the parasitoids (for total fecundity). The eggs daily removed from the glasses were maintained inside the same controlled environmental chambers under the controlled conditions until the emergence of the parasitoids.

Percent adult emergence from *C. cephalonica* and *H. armigera* eggs

Per cent emergence of *Trichogramma* adults was calculated from both i.e. *C. cephalonica* and *H. armigera* eggs by counting the host eggs in which there was an orifice intended for the exit of adults under a stereoscopic microscope, number of adults emerged per egg, calculated by dividing the total number of adults by the total number of orifices observed in each treatment [9]. Mean of all the three replications represented mean per cent emergence of *T. chilonis* adults form a given temperature treatment.

Total fecundity/female on *C. cephalonica* and *H. armigera* eggs

For recording the total fecundity of the adult females, a single mated female was kept in a glass tube along with 3 x 2 cm strips containing 40 eggs each from *C. cephalonica* and *H. armigera* separately for each treatment and the strips were replaced every day till the death of the female. The total number of eggs parasitized from these strips each day by the female were counted based on the changes in coloration of parasitized eggs for each treatment, suggesting the total lifetime fecundity.

Longevity of *T. chilonis* on *C. cephalonica* and *H. armigera*

After the emergence of the parasitoids individual females from both hosts were selected randomly and used in further experiments to determine the longevity of the adult females. The females were kept in flat-bottomed glass tubes, which contained a honey droplet on their inner surface for food and were plugged with cotton swab in three replicates. Each wasp was provided honey in equal amount, and so as to eliminate the effect of food level on how long the wasp survived in each treatment.

Sex ratio (F: M)

Adult sex ratio was determined by killing the emerged adults by keeping them under refrigerator at 0 ^{0}C. From those adults, male and females were separated by observing individual parasitoid under stereoscopic microscope based on the

differences in their morphological characters as given below:

Male: Males are darker and usually smaller than female, with black colour short, round abdomen. Antenna was distinct, long, more plumose, with black long bristles. Dorsum of thorax was brown with black tinge.

Female: Female are pale in colour and larger than male. Body was yellowish orange, with tapering abdomen. Antenna was short, not distinct, was less plumose with few short bristles.

Data analyses

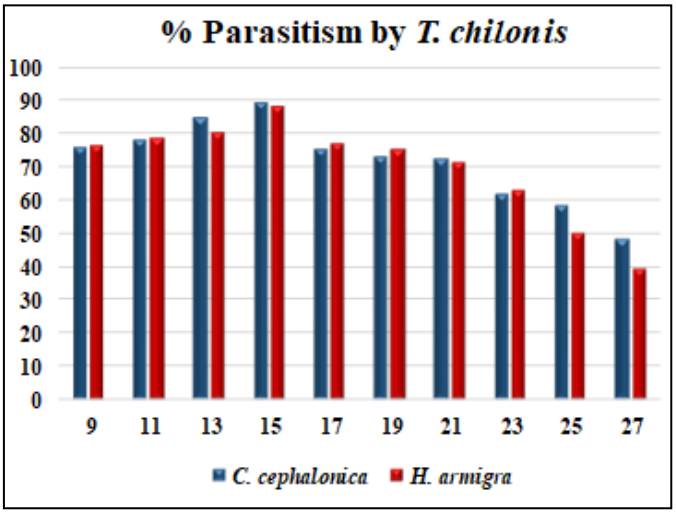
Data on the parasitism, emergence, fecundity, longevity and sex ratio of *T. chilonis* on the eggs of *C. cephalonica* and *H. armigera* was submitted to appropriate analysis [10]. The data that met these assumptions were then submitted to analysis of variance (ANOVA).

Results and Discussion

Our findings suggested the overall declining pattern with increase in temperature for the storage of the trichocards prepared form the both *C. cephalonica* and *H. armigera* eggs. Relatively lower temperatures are most favored by the *T. chilonis* as compared to high temperature.

Table 1: Means percent parasitism, emergence, total fecundity, sex ratio, and longevity of *Trichogramma chilonis* reared on *Corcyra cephalonica* and *Helicoverpa armigera* eggs at different temperatures.

Temperatures (^{0}C)	% Parasitism		% Adult emergence		Total fecundity/female		Adult longevity (Female)		Sex ratio (F:M)	
	C. cephalonica	*H. armigera*	*C. cephalonica*	*H. armigera*	*C. cephalonica*	*H. armigera*	*C. cephalonica*	*H. armigera*	*C. cephalonica*	*H. armigera*
9	76.00	76.67	81.67	83.00	83.67	84.00	11.50	11.17	1.67	2.33
11	78.33	78.67	84.67	83.33	88.67	84.33	9.67	10.00	2.00	3.00
13	85.00	80.33	85.33	88.33	92.33	90.67	8.83	8.67	5.00	4.00
15	89.33	88.33	94.00	96.00	99.33	103.67	8.00	8.17	2.00	2.33
17	75.67	77.33	86.33	85.00	86.00	90.67	7.50	7.50	2.50	1.40
19	73.33	75.33	78.00	74.33	83.33	83.00	7.33	7.17	2.00	2.67
21	72.67	71.67	69.67	73.00	77.33	80.33	6.67	6.67	3.00	1.00
23	61.67	63.33	62.00	62.33	74.67	76.33	5.83	5.83	1.00	1.50
25	58.67	50.33	49.33	57.67	70.33	69.00	5.33	5.00	2.50	1.33
27	48.33	39.33	45.67	47.67	66.33	65.00	4.17	4.33	2.50	1.67
S. Em. ±	1.650	1.174	1.130	1.211	1.633	2.074	0.230	0.167	0.298	0.219
C. D. at 5%	4.867	3.463	3.335	3.573	4.817	6.117	0.678	0.492	0.879	0.597
C. V. (%)	3.97	2.90	2.66	2.79	3.44	4.34	5.32	3.87	30.38	32.22



Graph 1. Showing per cent parasitism by *T. chilonis* on eggs of *C. cephalonica* and *H. armigera*, (Figures on X-axis indicates temperature; Y-axis indicates per cent parasitism).

Percent parasitism of the *T. chilonis* on *C. cephalonica* and *H. armigera*

The data presented on per cent parasitism of *T. chilonis* on the eggs of *C. cephalonica* and *H. armigera* in Table 1 (Column 3 & 4: Graph 1) showed that the treatment with the temperature of 15 ^{0}C was the most suitable temperature recording the highest per cent parasitism 89.33% in *C. cephalonica* and 88.33% in *H. armigera* as a host, suggesting the superiority of the treatment over others. With the recorded percent parasitism of 85.00% and 80.33%, respectively for *C. cephalonica* and *H. armigera* eggs treatment with 13 ^{0}C temperature was the second best after 15 ^{0}C. Following treatments were showing sharp decline in the percent parasitism by *T. chilonis* in both the hosts as the storage temperature increases. The least per cent parasitism was reported form the treatment with 27 ^{0}C temperature giving 48.33 and 39.33% parasitism in *Corcyra* and *Helicoverpa*, eggs respectively.

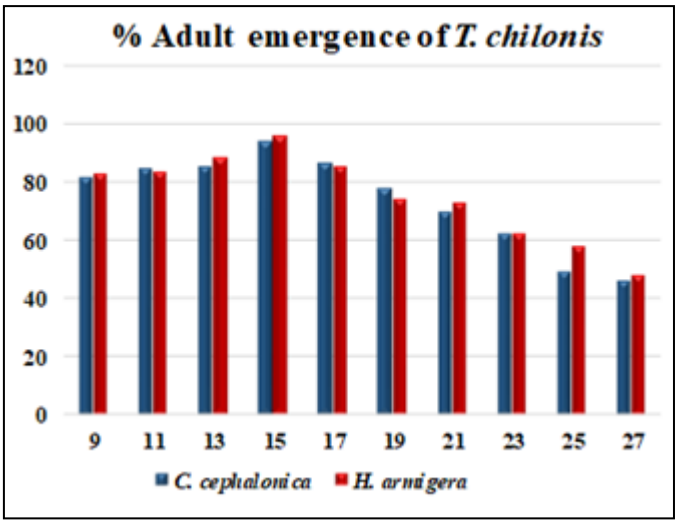
Current result is supported by the study of Mehendale (2009) [11] who reported that parasitized trichocards could be stored effectively up to 30 days at 15 ^{0}C and similar results were reported by Pathak, *et al*. in 2010 [12].

Percent adult emergence from *C. cephalonica* and *H. armigera* eggs

Table 1 (Column 4 & 5: Graph 2) shows the recorded adult emergence if *T. chilonis* from the parasitized eggs of *C. cephalonica* and *H. armigera*, suggesting again the superiority of the treatment with 15 ^{0}C storage temperature with 94% adult emergence in *C. cephalonica* eggs and 96% adult emergence in *H. armigera* eggs, in maintaining the viability of the parasitized eggs. *Helicoverpa* eggs showed slightly higher per cent emergence, this might be because of the larger size of the eggs. In the case of adult emergence second best treatment for *Corcyra* eggs was treatment 5 (17 ^{0}C) showing 86.33% emergence of *T. chilonis* adults followed by the treatment 3 (13 ^{0}C) which showed the 85.33% adult emergence. Whereas the reverse was true in case of Helicoverpa eggs where treatment 3 (13 ^{0}C) was second best by giving 88.33% adult emergence followed by the treatment 5 (17 ^{0}C) which recorded the 85.00% adult emergence. Rest

of the treatments after it were showing the declining trend of emergence with respective increase in temperatures in both the host with 27 ^{0}C treatment being giving the least recorded adult emergence (45.67 & 47.67% for *Corcyra* and *Helicoverpa*, respectively).

Present results were in accordance with the results of Bhargavi and Naik (2015) [13] who reported that the trichocards parasitized by *T. chilonis* could be stored effectively without much damage to the adult emergence up to 15 days at 15 ^{0}C, similar results were produced by Dileep in 2012 [14] who considered that the 15 ^{0}C temperature was suitable for the long term storage of the eggs parasitized by *T. chilonis*.

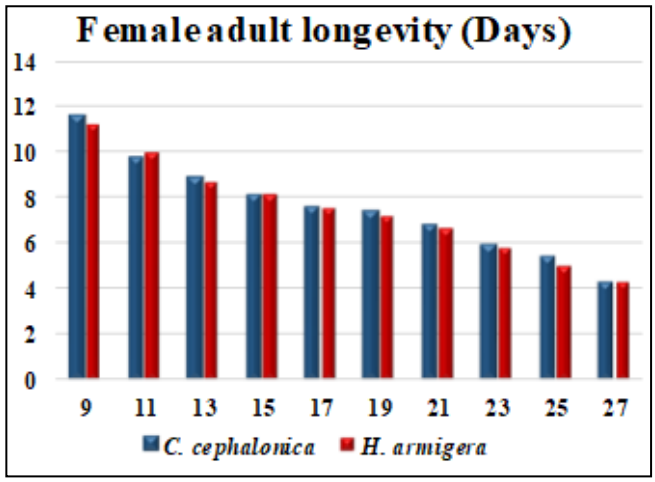


Graph 2: Showing per cent emergence of adults of *T. chilonis* on eggs of *C. cephalonica* and *H. armigera*, (Figures on X-axis indicates temperature; Y-axis indicates per cent adult emergence).

Total fecundity/female on *C. cephalonica* and *H. armigera* eggs

Total fecundity/female was recorded (Table 1, column 6 & 7: Graph 3) highest in case of treatment 4 i.e. in treatment with 13 ^{0}C storage temperature which has reported 103.67 parasitized eggs on the host *H. armigera*, same treatment was found most effective in case of *C. cephalonica* eggs with 99.33 parasitized eggs per female. Second best treatment in case of *Corcyra* eggs was treatment 3 (13 ^{0}C) reporting 92.33 parasitized eggs followed by the treatment with storage temperature 11 ^{0}C which showed 88.67 parasitized eggs. Whereas in case of *Helicoverpa* eggs second best treatments were treatments with 13^0 and 17 ^{0}C storage temperature which have recorded 90.67 parasitized eggs each. Inferiority of higher temperatures in giving the lower fecundity/ female was evident form the least performing treatment which was 27 ^{0}C storage temperature giving only 66.33 parasitized eggs from *Corcyra* and 65.00 from *Helicoverpa*.

A study conducted by Firake and Khan in 2014 [15] indicated that the exposure to higher temperatures drastically reduced the fecundity of the *T. chilonis*. Also it has been reported by number of workers [16-20] that high temperature shocks to pupae and adults have significant impacts on fecundity, life duration, mobility, and efficacy of *Trichogramma*.

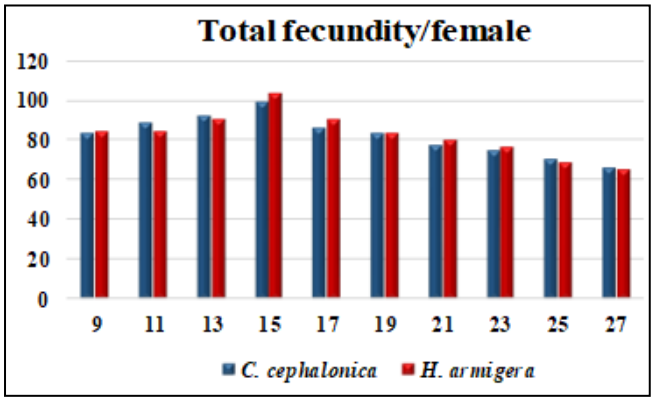


Graph 3: Showing the adult female longevity of *T. chilonis* on eggs of *C. cephalonica* and *H. armigera*, (Figures on X-axis indicates temperature; Y-axis indicates adult female longevity).

Longevity of *T. chilonis* females on *C. cephalonica* and *H. armigera*

Longest female longevity was recorded with the temperature of 9 ^{0}C which were 11.50 days for *C. cephalonica* and 11.17 days for *H. armigera*, and presented in Table 1 (column 8 & 9: Graph 4). Lower temperature seems to have profound effect on the longevity of the *Trichogramma* adults as they survive longer in case of the lower temperature as compared to the higher as suggested by the second best treatment which was 11 ^{0}C for both *Corcyra* and *Helicoverpa* eggs (9.67 and 10 days respectively). Longevity of the *T. chilonis* female was drastically affected by the increasing temperature as evident from the reduced longevity of the female exposed to treatments having higher temperatures, with treatment 10 i.e. 27 ^{0}C storage temperature recording the meager 4.17 days on *Corcyra* eggs and 4.33 days on *Helicoverpa* eggs.

A recent work by Carvalho *et al*. (2017) [21] on *T. pretiosum* reported the highest period of longevity (23.2 days) at 18 ^{0}C, which suggests that the results of current study are in accordance to their work as their study suggested lower temperature tends to increase the longevity of the adult due to reduced activity and it decreases with the increase in temperature.

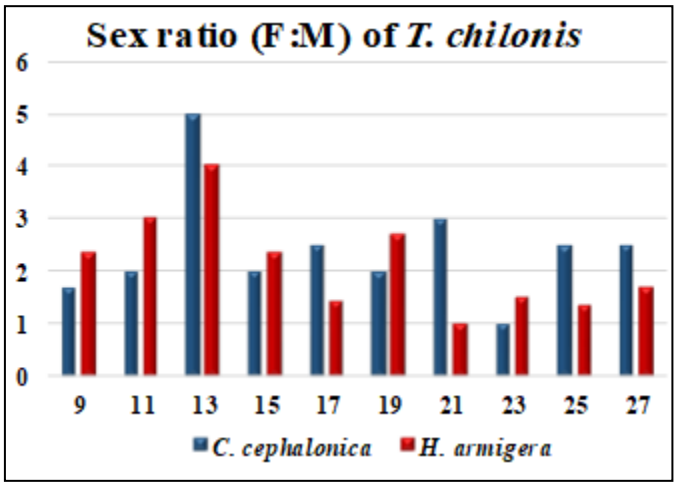


Graph 4. Showing total fecundity of *T. chilonis* female on eggs of *C. cephalonica* and *H. armigera*, (Figures on X-axis indicates temperature; Y-axis indicates total fecundity).

Sex ratio (F:M)

Data regarding female to male sex ratio of the *T. chilonis* adults is presented in table 1 (column, 10 & 11: Graph 5) showed higher female to male ratio (F:M) towards the temperature of the 13 ^{0}C, as it recorded the ratio of 5:1 (in *Corcyra* eggs), and 4:1 (in *Helicoverpa* eggs). In case of *Corcyra* eggs it was followed by the treatment with 21 ^{0}C which has recorded 3:1 female to male ratio. Whereas the *Helicoverpa* eggs recorded the same female to male ratio (3:1) in treatment involving 11 ^{0}C of storage temperature. Similar studies based on the effect of different temperatures on the sex ratio are not common.

Overall results suggests the temperature range of 13 ^{0}C to 15 ^{0}C can be the best storage temperature for the trichocards prepared from both the hosts. *C. cephalonica* and *H. armigera* both were found to be the good factitious host but based on the availability and the ease of multiplication *C. cephalonica* is the most obvious choice as a host for the mass production of the *T. chilonis*.



Graph 5: Showing the Female:Male (F:M) sex ratio of *T. chilonis* on eggs of *C. cephalonica* and *H. armigera*, (Figures on X-axis indicates temperature; Y-axis indicates adult female longevity).

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