



## Research Article

# Development of superior strain of *Trichogramma chilonis* Ishii for temperature tolerance

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**ABSTRACT:** Adaptation to climatic conditions (more importantly the temperature) of an area play a decisive role in influencing the parasitoid efficiency in controlling pests. Besides, the optimal conditions for inundative release of parasitoid e.g. *Trichogramma*, may also depend on strain. A strain of *Trichogramma chilonis* tolerant to high temperature was developed in the Biological control laboratory of IARI (PUSA), New Delhi and effect of temperature was studied on parasitoid efficiency. The biological traits viz., fecundity, emergence, survivability (male & female) and female populations were studied at 25, 27, 29, and 31°C. The fecundity and emergence were recorded highest at 27 °C. An increase of 107.23% (fecundity), 184.50% (emergence) and 15.65% (female survival) was observed from base to 35<sup>th</sup> generation, while male survival and female population decreased by 8.2 and 1.2%, respectively. The survival of immature stages of tolerant strain of *Trichogramma* proved their ability to withstand the temperature.

**KEY WORDS:** Emergence, fecundity, parasitoid, longevity, *Trichogramma*, sex ratio

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## INTRODUCTION

Parasitoids possess poor tolerance to certain climatic conditions like high temperature and low humidity. Poor parasitism, reduced emergence, male & female longevity and poor sex ratio reduces the efficiency of biological control agents. Smith (1996) observed that increasing the effectiveness of natural enemies may enhance the biological control action. *Trichogramma* is an most important biological control agent that has received maximum attention for the control of pests (King *et al.*, 1985). Wajinberg and Hassan (1994) reported that *Trichogramma* has been used against 28 different pests on 20 different crops. Inundative releases of *Trichogramma* were reported to be widely used for biological control of lepidopterous pests (Stouthamer *et al.*, 1999; Smith 1996; Hazarika *et al.*, 2009; Suckling and Brockerhoff 2010). Besides a few introduced species of *Trichogramma*, 12 indigenous species have been used in various release programme, and among these *T. chilonis* Ishii is widely distributed in Indian subcontinent. Infestation of stalk borer was significantly reduced with the release of *T. chilonis* (Tanwar *et al.*, 2003). However, poor

emergence, parasitization and poor sex ratio in many species have been observed in reducing their efficiency as biological control. Sugarcane borer was effectively controlled through the release of *T. chilonis* (Mustafa *et al.*, 2006). Singh *et al.* (2007) evaluated the genetically improved high temperature tolerant strain of *T. chilonis* against sugarcane early shoot borer. Effect of temperature on the immature development and emergence of *Trichogramma* was worked out by Foerster and Foerster (2009). Therefore, efforts were made to develop better strains of parasitoids with greater degree of tolerance to adverse climatic conditions which are of great importance in their practical application as biological control agents.

## MATERIALS AND METHODS

The study on development of superior strain of *Trichogramma chilonis* was conducted in the biological control laboratory of ICAR - Indian Agricultural Research Institute, (PUSA), New Delhi, India. These studies were conducted at 25, 27, 29 and 31° C in a BOD incubator. *Corcyra cephalonica* Stainton eggs parasitized by *T. chilonis* were

pasted individually on an egg card with diluted acacia gum and kept in separate vials. Fine streak of 50% honey solution was applied on the inner wall of the glass vial as adult food. Out of the newly emerged parasitoids, 25 pairs were made based on antennal variations. Each pair was kept in a homeopathic vial and its mouth was secured tightly with a plug. In each vial a small egg card bearing approximately 100 UV sterilized eggs was offered for parasitization on the first day of emergence. From the second day onwards, daily a fresh egg card containing approximately 50 eggs was offered to the parasitoid replacing the old card till the death of the female parasitoid. The fecundity of each female parasitoid was recorded separately by counting the number of eggs that turned black. Longevity of the parasitoids was recorded for observing the mortality of the parasitoid in each vial. Progeny of different highly fecund females were selected for crossing. The initial selection process was at 25°C up to 35<sup>th</sup> generation and then selection continued till the 50<sup>th</sup> generation. Initial selection for fecundity, longevity etc. was carried out at 25 °C till the 35<sup>th</sup> generation. After the 35<sup>th</sup> generation, the population was subjected to an increase in temperature up to 27 °C and selection continued at this temperature till the 40<sup>th</sup> generation. At 41<sup>st</sup> generation the selected population was exposed to further increase in temperature up to 29 °C and the selection continued till the 45<sup>th</sup> generation. At 46<sup>th</sup> generation, the population was again subjected to a temperature increase to 31°C and selection continued at this temperature up to the 50<sup>th</sup> generation. In each generation, the data on longevity of male and female were recorded by observing the mortality every day. Fecundity was calculated by recording the eggs parasitized by an individual parasitoid till its death. The number of adults emerged out of parasitized eggs were recorded sex wise and the percentage of females emerged in each generation was worked out. For developing a superior strain of *T. chilonis*, a population of the species collected from sugarcane ecosystem was selected up to 35<sup>th</sup> generation for improving the fecundity, emergence, longevity and sex ratio. After 35<sup>th</sup> generation, this population was exposed to gradual increase in temperature at three successive increasing temperatures i.e. 27, 29 and 31 °C. The *T. chilonis* was reared at a given temperature for five generations performing selection at each generation.

## RESULTS AND DISCUSSION

### Effect of temperature on biological attributes

The results obtained on mean fecundity, emergence, male longevity, female longevity and per cent females in each generation of *T. chilonis* and day wise egg laying pattern of *T. chilonis* are presented in Table 1 & Table 2, respectively and % increase and decrease of biological

attributes is depicted in Fig 1. The selective breeding up to 35<sup>th</sup> generation was done on populations having higher fecundities, and it was observed that the selective breeding resulted in the increase in fecundity from base generation to 35<sup>th</sup> generation. (Table 1 and Fig. 1). An increase in temperature up to 27 °C resulted in increased fecundity from initial (36<sup>th</sup> generation) to final (40<sup>th</sup> generation) by 11.46% and further increase in temperature up to 29°C (41<sup>st</sup> generation to 45<sup>th</sup> generation) resulted in increased fecundity by 30.48 and 5.43%, respectively. Mean fecundity of mated female was very high in the temperature range of 25 to 27°C, as the egg laying was staggered over a longer period of time. The selective breeding increased the emergence from base generation to 35<sup>th</sup> generation by 184.50%. Temperature increase up to 27° C resulted in increased emergence from initial (36<sup>th</sup> generation) to final (40<sup>th</sup> generation) by 6.18% and further increase in the temperature up to 29° C (41<sup>st</sup> generation to 45<sup>th</sup> generation) to 31° C resulted in increased emergence by 33.60 and 5.69%, respectively.

A decrease in male survivability from base generation to 35<sup>th</sup> generation by 8.2% was observed. Temperature increase up to 27 °C decreased male survivability by 3.95%. Further increase in temperature up to 29°C (41<sup>st</sup> generation to 45<sup>th</sup> generation) recorded increase in male survivability by 62.71%, whereas further increase in temperature up to 31 °C, decreased the male survivability by 27.78% (Fig. 1). It was observed that female survivability increased from base generation to 35<sup>th</sup> generation by 15.65%. Increased temperature up to 27°C resulted in female survivability from initial (36<sup>th</sup> generation) to final (40<sup>th</sup> generation) by 0.62%, further increase up to 29°C (41<sup>st</sup> generation to 45<sup>th</sup> generation) and 31°C resulted in increase in female survivability by 26.60 and 7.29%, respectively.

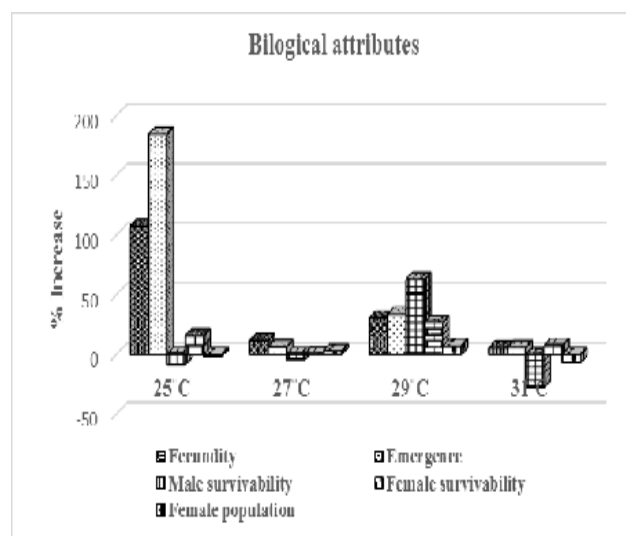


Fig. 1. Increase/decrease in biological attributes with varying temperature.

**Table 1. Development of temperature tolerant strain of *Trichogramma chilonis***

Temperature	Generation	Fecundity (%)	Emergence (%)	Male survivability (Days)	Female survivability (Days)	Female population (%)
25 °C	Base (Initial)	44.28	21.93	02.92	05.88	40.28
	35 <sup>th</sup> (Final)	91.76	62.39	02.68	06.80	39.78
	Mean*	77.54	62.71	03.00	06.11	45.02
	S.E. (m) ±	05.65	04.39	00.37	00.48	03.43
27 °C	36 <sup>th</sup> (Initial)	82.00	65.40	03.04	06.48	46.53
	40 <sup>th</sup> (Final)	91.40	69.44	02.92	06.52	47.82
	Mean**	81.02	66.87	02.95	06.01	47.56
	S.E. (m) ±	04.38	04.39	00.19	00.34	10.77
29 °C	41 <sup>st</sup> (Initial)	47.24	39.40	00.59	03.76	44.62
	45 <sup>th</sup> (Final)	61.64	52.64	00.96	04.76	47.78
	Mean***	62.80	53.86	02.20	04.14	46.98
	S.E. (m) ±	03.49	03.41	00.14	00.25	10.02
31 °C	46 <sup>th</sup> (Initial)	67.76	52.76	00.90	03.84	52.17
	50 <sup>th</sup> (Final)	71.44	55.76	00.65	04.12	48.84
	Mean****	62.80	61.15	02.57	03.91	48.71
	S.E.(m) ±	04.02	03.01	00.16	00.25	07.70

\*Mean for base to 35<sup>th</sup> generation\*\* Mean for 36<sup>th</sup> to 40<sup>th</sup> generation\*\*\*Mean for 41<sup>st</sup> to 45<sup>th</sup> generation\*\*\*\*Mean for 46<sup>th</sup> to 50<sup>th</sup> generation

Data presented in Table 1 also reveals that selective breeding resulted in decrease of female population from base generation to 35<sup>th</sup> generation by 1.2%. An increase in temperature up to 27°C recorded increased female population from initial (36<sup>th</sup> generation) to final (40<sup>th</sup> generation) by 2.77 % and further increase in temperature up to 29°C (41<sup>st</sup> to 45<sup>th</sup> generation) increased female population by 7.08%, while further increase up to 31°C, decreased female population by 6.38%.

#### Egg Laying Pattern of Female *Trichogramma chilonis*

It was observed that more than 50% of the total number of eggs laid by a female were deposited on the first day of oviposition in most of the generations. A gradual reduction in the number of eggs laid on the subsequent days was observed. On the last day only about 1% of the total number of eggs were laid (Table 2). The parasitoids reared at the optimum temperature range of 25 to 27 °C, the egg laying was staggered over a longer period of time *i.e.* up to 11 days, but when temperature was increased to 29°C and 31°C, the egg laying on the first day showed an appreciable increase and the total egg laying was completed in 5 and 4 days, respectively.

By and large increase in all the parameters related to biological attributes could be due to selective breeding of the population. After successive generations of exposure to

temperature, the sequential increase in temperature resulted in development of a strain with tolerance to the higher temperatures. With increase in temperature, there was an increased tendency of oviposition, so as to perpetuate the progeny. Inbreeding contributed to a considerable amount of homogeneity in the selected population. The increase in fecundity by selection may be due to predominance of the additive gene action. Similar evaluation of genetically improved strain of *T. chilonis* for the management of sugarcane stalk borer (*Chilo auricilius*) was worked out by Singh *et al.* (2008). The increase in mean fecundity largely depends upon the amount of genetic variability present in the culture undergoing selection and predominance of epistatic and dominance type of gene action. Possible reasons for decreased fecundity at higher temperature could be attributed to the reduction in longevity of the female parasitoid or partial sterility of females due to high temperature. The other possible reason could be occurrence of correlated, pleiotropic variations of deleterious nature during selection. Overall increase in fecundity obtained at the end of the selection may be due to dominant effect of additive genes. Pak *et al.* (1985) also reported that in all three strains of *Trichogramma* tested at 12, 17, 20, 25 & 30°C, parasitism increased with increasing temperature to a maximum at 20 to 25°C and declined at 30°C. The significant improvement in emergence may be due to selective breed-

ing of the population, even when the rearing temperature was enhanced. Similarly Dadmal *et al.* (2010) reported the influence of short term exposure to different temperatures of *T. chilonis* Ishii. However, adult emergence, number of parasitized eggs per female and female longevity were adversely affected when the egg and larval stages of *T. chilonis* were exposed to higher temperatures beyond  $30\pm 1^{\circ}\text{C}$  and  $40\pm 1^{\circ}\text{C}$ , however the pupal stage was found relatively tolerant. Many studies have shown that the adult survivability of *Trichogramma* decreases with increasing temperature (within thermal tolerance range) (Cabello and Vargas 1988; Consoli & Parra 1995; Mc Dougall and Mills 1997; Schollen and Hassan, 2001, Ayvaz *et al.*, 2008). High mortality rates of *Trichogramma* at high temperature by and large can be overcome by organizing successive emergence

waves (Fradon *et al.*, 2002). Influence of temperature, during fecundity development has been studied by numerous workers, however parasitism rates were highest at  $20^{\circ}\text{C}$  for many species (Pavlik, 1992). Within a certain period of time, highest number of hosts were parasitized at  $25^{\circ}\text{C}$  (Cabello and Vargas 1988; Consoli & Parra 1995; Mc Dougall and Mills 1997; Schollen and Hassan 2001, Ayvaz *et al.*, 2008). The potential rate of increase or the ability to outnumber the host is recognized as a very important attribute of an effective natural enemy, especially in variable environments. The natural enemies having these qualities can overtake their hosts quickly whenever the host begins to increase in numbers. This developed strain can be used as a component of bio-intensive IPM in various crops, where temperature goes higher.

**Table 2. Day wise egg laying pattern of *Trichogramma chilonis***

		Mean Fecundity (Day wise )													
Generation	Rearing temp.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Base	25 °C	26.80	8.56	2.76	3.08	1.24	0.92	0.40	0.48	0.04	-	-	-	-	-
1	25 °C	35.88	9.40	4.72	1.72	0.80	0.48	0.12	-	-	-	-	-	-	-
2	25 °C	30.64	12.24	6.32	1.88	-	-	-	-	-	-	-	-	-	-
3	25 °C	39.32	10.72	0.72	0.16	0.08	-	-	-	-	-	-	-	-	-
4	25 °C	24.96	25.36	12.00	10.92	0.32	-	-	-	-	-	-	-	-	-
5	25 °C	40.00	18.22	9.83	4.74	2.78	0.65	0.39	0.09	-	-	-	-	-	-
6	25 °C	43.04	22.21	8.42	4.54	2.58	1.58	0.71	0.08	-	-	-	-	-	-
7	25 °C	42.72	14.92	13.84	6.64	2.16	1.04	0.24	0.08	-	-	-	-	-	-
8	25 °C	32.64	12.21	4.21	2.14	0.79	0.29	-	-	-	-	-	-	-	-
9	25 °C	29.21	4.21	0.13	-	-	-	-	-	-	-	-	-	-	-
10	25 °C	43.28	4.92	7.88	-	-	-	-	-	-	-	-	-	-	-
11	25 °C	21.08	12.72	9.28	5.12	3.16	0.68	0.08	-	-	-	-	-	-	-
12	25 °C	45.00	20.90	12.04	5.92	4.84	2.76	1.36	0.64	-	-	-	-	-	-
13	25 °C	50.12	27.60	12.00	6.48	4.40	2.40	2.48	0.72	0.52	-	-	-	-	-
14	25 °C	48.76	14.12	8.44	4.60	2.40	1.12	0.32	0.04	-	-	-	-	-	-
15	25 °C	49.32	24.88	10.16	5.56	3.68	2.52	0.60	0.12	-	-	-	-	-	-
16	25 °C	45.08	19.32	8.64	8.36	5.00	2.92	2.12	1.76	0.84	0.52	0.40	-	-	-
17	25 °C	43.16	18.80	11.64	2.48	0.96	0.32	0.12	0.08	-	-	-	-	-	-
18	25 °C	61.68	16.68	7.64	5.52	2.20	1.24	0.36	0.12	-	-	-	-	-	-
19	25 °C	65.28	17.44	9.80	3.48	1.60	0.56	0.04	-	-	-	-	-	-	-
20	25 °C	55.12	16.40	8.08	3.08	1.60	0.80	0.24	0.08	-	-	-	-	-	-
21	25 °C	61.28	20.92	10.40	6.04	3.44	0.92	0.16	0.04	-	-	-	-	-	-
22	25 °C	57.28	21.96	14.36	8.20	4.40	1.88	0.56	0.08	-	-	-	-	-	-
23	25 °C	53.72	19.27	11.26	8.30	1.60	0.35	0.25	0.05	-	-	-	-	-	-
24	25 °C	49.70	18.23	10.79	8.90	1.92	0.23	0.21	0.12	-	-	-	-	-	-
25	25 °C	40.72	17.20	9.60	7.04	3.92	2.08	0.76	0.28	-	-	-	-	-	-
26	25 °C	42.20	14.08	7.16	4.12	2.44	1.44	1.08	0.32	0.20	-	-	-	-	-
27	25 °C	53.80	18.36	7.52	5.48	2.00	1.60	1.08	0.64	0.36	0.12	-	-	-	-
28	25 °C	37.36	13.08	6.04	3.48	2.28	1.32	0.40	-	-	-	-	-	-	-
29	25 °C	52.72	21.00	10.04	5.56	4.24	2.84	1.96	1.52	0.76	0.08	-	-	-	-

30	25 °C	47.68	16.80	6.84	4.72	2.48	1.44	0.72	0.24	-	-	-	-	-	-
31	25 °C	45.56	15.88	15.20	7.68	5.0	3.60	1.60	1.00	0.40	-	-	-	-	-
32	25 °C	34.00	17.16	11.16	10.88	5.64	4.24	1.36	0.48	0.36	0.16	-	-	-	-
33	25 °C	31.48	15.40	8.72	4.32	2.76	1.96	1.52	0.76	0.08	-	-	-	-	-
34	25 °C	35.40	14.52	7.56	2.52	1.40	0.76	0.12	0.04	-	-	-	-	-	-
35	25 °C	53.56	19.64	10.92	5.12	3.04	1.56	0.36	0.12	-	-	-	-	-	-
36	27 °C	44.68	13.00	15.80	4.16	2.44	0.92	0.68	0.32	-	-	-	-	-	-
37	27 °C	51.40	17.20	8.56	4.52	1.64	0.40	-	-	-	-	-	-	-	-
38	27 °C	45.36	13.32	6.80	2.96	2.08	1.56	1.00	0.44	-	-	-	-	-	-
39	27 °C	48.20	15.76	7.36	3.12	0.52	-	-	-	-	-	-	-	-	-
40	27 °C	44.24	30.72	10.24	4.52	1.64	0.44	0.08	-	-	-	-	-	-	-
41	29 °C	31.12	10.84	5.28	-	-	-	-	-	-	-	-	-	-	-
42	29 °C	52.92	13.08	0.20	-	-	-	-	-	-	-	-	-	-	-
43	29 °C	47.72	23.20	2.44	0.56	-	-	-	-	-	-	-	-	-	-
44	29 °C	41.20	18.56	4.24	1.40	0.84	0.56	-	-	-	-	-	-	-	-
45	29 °C	36.68	17.64	5.92	1.08	0.32	-	-	-	-	-	-	-	-	-
46	31 °C	41.60	21.08	5.16	-	-	-	-	-	-	-	-	-	-	-
47	31 °C	64.72	23.52	3.96	0.52	-	-	-	-	-	-	-	-	-	-
48	31 °C	39.88	17.12	3.28	0.56	-	-	-	-	-	-	-	-	-	-
49	31 °C	54.68	14.88	2.68	0.24	-	-	-	-	-	-	-	-	-	-
50	31 °C	51.00	17.52	2.76	0.16	-	-	-	-	-	-	-	-	-	-

The tolerant strain developed showed an increase in biological attributes from base generation to 35<sup>th</sup> generation by 107.23% (fecundity), 184.50% (emergence), 15.65% (female survivability), whereas male survivability and female population decreased by 8.2% and 1.2%, respectively. Optimum range of egg laying was when temperature was between 25 and 27°C. Better strains of parasitoids with greater degree of tolerance to adverse climatic conditions are of great importance in their practical application as biological control agents.

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