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Comparative assessment of host selection behavior of *Trichogramma* species under no-choice and dual-choice conditions

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Abstract

Trichogramma chilonis Ishii (Hymenoptera: Trichogrammatidae), which is typically raised on *Corcyra cephalonica* eggs, is used extensively in the biological control of lepidopteran pests. However, research into alternate hosts like the Eri silkworm, or *Philosamia ricini*, may be beneficial for integrating with current agro-silkworm systems and achieving sustainable mass production. Utilising eggs from *C. cephalonica* and *P. ricini* over five consecutive generations, this study assessed the host selection behaviour and biological performance of *T. chilonis* under no-choice and dual-choice conditions. Whereas dual-choice tests replicated field conditions by simultaneously exposing parasitoids to both hosts, no-choice tests evaluated host suitability by exposing them to only one host type per replicate. The percentage of parasitisation, adult emergence per egg, female progeny percentage, and adult longevity were among the biological parameters that were assessed. The findings showed that although the first generations preferred *C. cephalonica*, *T. chilonis* performance was greatly enhanced by repeated exposure to *P. ricini* in every parameter, suggesting behavioural adaptation and physiological compatibility. Eri eggs may make an excellent long-term host due to their improved reproductive characteristics and survivability. The use of Eri eggs has applications in sericulture that go beyond entomological considerations. The production of eri eggs will provide farmers with value addition and a source of income diversification. Their use in *Trichogramma* mass rearing offers a low-cost, high-return model that supports rural livelihoods and pest management. The study emphasises that *P. ricini* eggs are a viable, environmentally friendly substitute host for large-scale *T. chilonis* rearing, encouraging cooperation between biological control and sericulture efforts.

Keywords: *Trichogramma*, host preference, no-choice test, dual-choice test, *Philosamia ricini*

1. Introduction

The tiny egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) is used extensively in a variety of agroecosystems as a biocontrol agent for lepidopteran pests (Wang *et al*., 2016) [19]. A sustainable and environmentally responsible method of managing pests, biological control lessens reliance on synthetic pesticides, which frequently result in resistance, residue build-up, and ecological imbalance. Due to their widespread use against lepidopteran pests, egg parasitoids of the genus *Trichogramma* have attracted a lot of attention among biological control agents (Romeis *et al*., 1997; Wang *et al*., 2016) [13, 19]. Because of its versatility, quick reproduction, and potency against a variety of pests, such as *Helicoverpa armigera*, *Spodoptera litura*, and *Chilo partellus*, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) is one of the most commonly used species throughout Asia, particularly in India (Mohd and Mahla, 2023; Ara, 2015; Li *et al*., 2024) [11, 4, 21].

To increase effectiveness of *T. chilonis* in biocontrol programs, it is essential to comprehend its host preference behaviour. Two experimental conditions are commonly used to evaluate host preference: dual-choice tests, in which parasitoids are simultaneously presented with two host types, and no-choice tests, in which they are exposed to only one host type (Chunke and Shankar, 2017) [1]. These experimental configurations provide insights into host selection behaviour under both restricted and natural-like conditions, aiding in the differentiation between host acceptability (no-choice) and host preference (dual-choice) (Romeis *et al*., 1997) [13].

T. chilonis is frequently produced in large factitious hosts such as the eri silkworm (Samia cynthia ricini) and the Chinese oak silkworm (*Antheraea pernyi*, COS). Due to their size and accessibility, COS eggs have been preferred; however, they have drawbacks, including limited worldwide availability, variable parasitism and emergence rates, and thick chorions that hinder emergence (Zhang *et al*., 2024; Consoli *et al*., 2010) [22, 2]. On the other hand, ES eggs have benefits like thin eggshells, cost-effectiveness, rapid development, and broad distribution (Zhang *et al*., 2024; Yuan *et al*., 2024) [22, 21].

The host selection process is one of the most important aspects of the parasitoid-host interaction. Key biological traits of *Trichogramma*, such as fecundity, emergence rate, sex ratio, adult longevity, host searching behaviour, host preference for particular target species, and adaptability to local climate conditions, all have a significant impact on this process. The parasitism process consists of five sequential steps: host habitat location, host location, host acceptance, host suitability, and host regulation (Flanders, 1937; Gordh *et al*., 1999; Vinson, 1976) [5, 6, 18]. Generally speaking, the first three phases are regarded as a component of the host selection process, which may entail selecting between different host species or developmental stages.

On the other hand, host suitability concentrates on the elements that affect the ability of the parasitoid to develop successfully within a particular host. Successful parasitism is ultimately determined the capacity of parasitoid to finish developing inside its host after it has found and chosen one (Pak *and* van Heiningen 1990) [12].

Environmental factors, as well as host-specific characteristics like chemical and physical cues, egg size, shape, and age, can all have an impact on host selection. To evaluate the parasitism behaviour of various *Trichogramma* species or strains on either factitious host eggs or target pest eggs, preference tests are frequently carried out for use in biological control programs (van Dijken *et al*., 1986) [17]. Nevertheless, because different host eggs might not coexist on the same crop or appear at the same time, such evaluations might not always accurately represent real-field dynamics (Samara *et al*., 2011) [14]. The need for more ecologically relevant host preference testing is highlighted by this limitation.

Eri silkworm is multivoltine, non-dormant insect makes an appropriate artificial host egg for *Trichogramma parasitism*. This is because of its benefits, which include low cost, high resistance, short developmental period, and high reproduction rate. A few studies discovered that *Trichogramma dendrolimi*, *Trichogramma confusum*, and *T. chilonis* are raised from ES eggs. Thus, to guarantee a sufficient number of eggs for the rearing of egg parasitoids, a sufficient population of female moths must be maintained throughout the year.

T. chilonis is compelled to parasitise the provided host in no-choice tests, regardless of its suitability. This condition mainly assesses host acceptance and offers important insights into developmental success and physiological compatibility. Dual-choice tests, on the other hand, mimic field conditions by presenting the parasitoid with two hosts at the same time, thereby exposing its true preference or behavioural inclination (Mohd and Mahla, 2023) [11]. The best host for mass rearing, as well as the likelihood of successful establishment and performance in field applications, are all determined by these comparisons.

The differences in *T. chilonis* parasitism efficiency, emergence rate, sex ratio, and adult longevity among various hosts have been described in a number of studies. As an example, Mohd and Mahla (2023) [11] showed that *T. chilonis* significantly outperformed *Helicoverpa armigera* in parasitising *Corcyra cephalonica* eggs, especially when the eggs were 8 hours old. Chunke and Shankar (2017) [1] also noted that the eggs of *C. cephalonica* had better female progeny ratios and adult emergence, making them more suitable for parasitism. According to these results, parasitoid performance is highly influenced by host age and species, and these aspects need to be taken into account when choosing hosts for field deployment and rearing.

Numerous factors, such as host egg size, age, surface chemistry, and nutritional content, affect host preference in *T. chilonis*. Large, fresh, and viable eggs are frequently preferred by females because they provide the developing larvae with superior nutrition and survival chances (Romeis *et al*., 1997) [13]. Furthermore, parasitoids can distinguish between different hosts by detecting chemical and physical cues from their surfaces. Wang *et al*. (2016) [19] discovered that *T. chilonis* females avoid previously parasitised or low-quality hosts by evaluating egg suitability using ovipositor probing and antennal drumming.

Trichogramma species have been able to multiply in large numbers in recent years due to the move towards assessing alternative and factitious hosts, like the eri silkworm *Philosamia ricini* (Shi *et al*., 2022) [15]. According to these studies, bigger hosts like *P. ricini* support higher levels of parasitism and emergence while also improving the fitness of their offspring. However, a great deal of research is needed to evaluate behavioural compatibility and preference in both no-choice and dual-choice scenarios because such hosts are novel.

The purpose of this study is to assess host preference of *Trichogramma chilonis* in both no-choice and dual-choice tests for a variety of host species and egg ages. In order to assess host suitability, the study focusses on important factors like longevity, sex ratio, emergence rate, and parasitisation percentage. It is anticipated that these results will help determine the best hosts for mass rearing and increase efficacy of *T. chilonis* in integrated pest management initiatives.

2. Materials and Methods

2.1 Host Cultures and Rearing Conditions

Rice moth (*Corcyra cephalonica*) and eri silkworm (*Philosamia ricini*) cultures were kept in the Division of Sericulture at SKUAST-Jammu under carefully monitored laboratory conditions (27±1 °C, 70±5% RH, 12:12 L).

Sanitation: To get rid of contaminants, rooms and equipment were cleaned with 2% formalin before being reared and sealed for 48 hours.

2.2 Host preference of *Trichogramma* species under No-choice test and Dual Choice test

The host preference of *Trichogramma* species; *T. chilonis* were evaluated using freshly laid eggs of *Corcyra cephalonica* (rice moth) and *Philosamia ricini* (Eri silkworm). Two bioassays were conducted: a no-choice test to assess host suitability and a dual-choice test to evaluate host preference. Each experiment was conducted for five generations (F_0, F_1, F_2, F_3, F_4), with five replications per treatment.

Daily collections of fresh *Corcyra cephalonica* and eri silkoworm eggs (less than 24 hours old) were processed for parasitisation in accordance with protocol by Ghosh and Ballal, 2017 and Lalita *et al.*, 2010 [9, 23]. Gum Arabic was used to adhere the UV-treated eggs to paper cards after they had air dried.

2.2.1 No-choice Bioassay

T. chilonis was examined independently on either Eri or *Corcyra* eggs in the no-choice test. 50 recently emerged adult *Trichogramma* were added to glass vials along with cards containing 100 eggs of a single host species. Under a 12:12 (L:D) photoperiod, the parasitoids were permitted to parasitise the host eggs for a full day at 27±1 °C and 70±5% relative humidity. With the same host species being used for parasitisation in each subsequent generation (F0, F_1, F2, F3, and F4), parasitoids that emerged from each host were utilised to perpetuate the corresponding lineage. This method was used to investigate trends in parasitoid performance over time and host suitability when raised solely on one host.

2.2.2 Dual-Choice Bioassay

Vials were provided with mixed cards of 50 eggs per host species (ES and *C. cephalonica*). Fifty parasitoid adults were introduced and allowed to oviposit for 24 h under identical environmental conditions. After exposure, the two host types were separated and incubated independently. Parasitoids emerging from ES eggs were used to establish the next generation, ensuring generational tracking of choice adaptation.

The following biological parameters were recorded for each replicate across all generations: percentage parasitism, number of adults emerged per egg, female emergence percentage, and adult longevity. In accordance with developmental timing reported for large factitious hosts (Zhang *et al.*, 2024) [22], parasitism and emergence were evaluated approximately 10-12 days after exposure.

3. Results and Discussion

The parasitism success, female emergence, and longevity of *Corcyra cephalonica* eggs and *Philosamia ricini* (Eri silkworm) eggs were found to differ significantly when the host selection behaviour of *Trichogramma chilonis* was compared over five generations under no-choice and dual-choice conditions. According to Zhang *et al*. (2024) [22], who highlighted the crucial role that host suitability and host conditioning play in determining parasitoid efficacy, these variations underscore the influence of host size, quality, and adaptability on parasitoid biology.

3.1 Percent parasitism under No-Choice and Dual-choice Test

There were notable variations in the parasitism success and progeny performance of the hosts under no-choice conditions, where *T. chilonis* was only allowed to parasitise one host species per replicate (Table 4.1.1). The parasitisation rate of 96.60± 0.50 percent on *C. cephalonica* was notable for the first generation (F_0), and it was significantly higher than the 84.20±0.66 percent on *P. ricini*. While *Corcyra* showed a decreasing trend from F_1 onwards, reaching 88.60± 0.50 percent by F_4, the parasitism percentage on Eri eggs increased over generations, peaking at 92.20±0.66 percent in F_4. The greater initial parasitisation rate on *Corcyra* eggs under no-choice test points to an innate or preconditioned preference for the conventional host. This pattern is in line with earlier research that found that *T. chilonis* prefers *C. cephalonica* as a host because of its extensive use in mass rearing programs (Romeis *et al*., 1997; Mohd and Mahla, 2023) [13, 11].

4.1.1: percent parasitism of *T. chilonis* reared on eri silkworm eggs and rice moth eggs under no-choice and dual-choice test

Generations	No-choice		Dual-choice	
	Eri	*Corcyra*	Eri	*Corcyra*
F_0	84.20±0.66a	96.60±0.50b	48.40±3.05a	90.40±1.46b
F_1	81.00±0.44a	97.60±0.24b	64.80±2.65a	88.80±1.85b
F_2	84.00±0.70a	95.20±0.73b	78.00±3.52a	84.40±2.13b
F_3	89.00±1.00a	91.60±0.81a	85.60±1.16a	80.40±1.16a
F_4	92.2±0.66a	88.60±0.50a	90.00±1.26b	76.80±1.95a
SEm+	0.71	0.59	2.52	1.75

Values are Means±SE

Means in a row followed by different letters are significantly different between two different hosts (Student's t-test, $p<0.05$)

T. chilonis was able to exhibit innate preference behaviour in the dual-choice test because both host types were presented at the same time (Table 4.1.2). A greater number of eggs were parasitised on *Corcyra* in the early generations, particularly F_0 and F_1 (45.20±0.73 and 44.40±0.92, respectively), suggesting an early behavioural preference for the conventional host. A change in preference across generations was evident, though, as the preference flipped by F_4, with 45.00±0.63 parasitised Eri eggs compared to 38.40±0.97 *Corcyra*.

This generational adaptation was also reflected in the parasitisation percentage. Initially, *Corcyra* was higher (90.40±1.46 percent in F_0), but Eri eggs were parasitised by F_4 at a rate of 90.00±1.26 percent, which was higher than *Corcyra* (76.80±1.95%). This change suggests that host preference and recognition were improved by selection pressure and repeated exposure to Eri eggs.

However, the observed generation-to-generation increase in parasitism percentage on *P. ricini* from F_0 to F_4 shows that *T. chilonis* can gradually adapt to new hosts, particularly when host quality promotes successful development. Similar patterns were noted by Zhang *et al*. (2024) [22], who found that *T. chilonis* parasitism rates on *Samia cynthia ricini*; a close relative of *P. ricini* surpassed those on *Antheraea pernyi* over several generations, demonstrating the behavioural flexibility and abilityof the parasitoid to adapt to its host. Moreover, host acceptance is a crucial stage after host recognition that is heavily impacted by both innate host suitability and past conditioning, according to Flanders (1937) and Vinson (1976) [5, 18].

3.2 Adult emerged per egg under No-Choice and Dual-choice Test

Interestingly, on *P. ricini*, the number of adults that emerged per egg was consistently and significantly higher. In the F_4 generation, *T. chilonis* multiplied on Eri produced 6.60 ± 0.40 adults per egg, while those raised on *Corcyra* only produced 1.20 ± 0.20. Emergence from Eri eggs showed a consistent upward trend from F_1 to F_3, suggesting higher host utilisation efficiency (Wang *et al*., 2020; Hou *et al*., 2022) [20, 7]. (Table 4.1.1). Its better physiological compatibility is suggested by consistent superiority of *P. ricini* in supporting higher adult emergence per egg (rising to 8.60 in F_4 under no-choice). On the other hand, in every generation, the number of adults per *Corcyra* egg was limited to a single emergence.

Eri eggs continuously produced more adults per egg throughout the dual-choice test. In F_0, the emergence was 6.00 ± 0.31, but in F_4, it was 8.00 ± 0.17. By comparison, *Corcyra* produced only one adult per egg in every generation, demonstrating the higher physiological suitability of Eri eggs.

4.1.2: Number of adults emerged per egg of *T. chilonis* reared on eri silkworm eggs and rice moth eggs under no-choice test

Generations	No-choice		Dual-choice	
	Eri	***Corcyra***	**Eri**	***Corcyra***
F_0	6.60±0.40b	1.20±0.20a	6.00±0.31b	1.00±0.00a
F_1	7.00±0.44b	1.00±0.00a	6.60±0.24b	1.00±0.00a
F_2	7.40±0.24b	1.00±0.00a	7.20±0.20b	1.00±0.00a
F_3	8.20±0.37b	1.00±0.00a	7.60±0.00b	1.00±0.00a
F_4	8.60±0.24b	1.00±0.00a	8.00±0.17b	1.00±0.00a
SEm+	0.35	0.28	0.22	0.00

Values are Means±SE

Means in a row followed by different letters are significantly different between two different hosts (Student's t-test, $p<0.05$)

The smaller egg size of *C. cephalonica* may be the cause of this restriction, which limits the number of larvae that can successfully develop inside a single egg.

These results corroborate earlier research by Consoli *et al.* (2010) [2], who observed that larger host eggs facilitate better larval survival and adult emergence in addition to allowing for higher egg loads. According to Pak *et al.* (1990) [12], the number of viable progenies is also significantly influenced by the size and nutritional value of the host, particularly in egg parasitoids like *Trichogramma* species. The enhanced emergence pattern suggests that *P. ricini* eggs have more room and nutrients, as well as advantageous physical characteristics after KOH treatment, which probably helped the larvae grow. Li *et al.* (2018) [10] found that manually softened *Antheraea pernyi* eggs had higher emergence rates, which supports this finding.

Such increased development efficiency in larger hosts is consistent with Vinson's (1976) [18] physiological suitability model, which states that parasitoid success is greatly influenced by the volume and internal chemistry of the host egg.

3.3 percent female emergence under No-Choice and Dual-choice Test

The percentage of female emergence (Table 4.1.3) showed a dynamic change over generations. *Corcyra* had a higher percentage at first (63.73±1.23 percent in F_0), but by F_4, it had dropped to 51.76± 1.60 percent. In contrast, female emergence from Eri eggs showed improved host adaptation, increasing over time to 67.68± 0.64 percent in F_4. *Trichogramma* sex allocation is directly influenced by host quality, according to Wang *et al.* (2016) [19], with higher-quality hosts producing a larger percentage of females. Consoli *et al.* (2010) [2] also verified that the chorion permeability and nutritional sufficiency o host egg impact the results of sex ratio and embryonic viability.

4.1.3: percent female emergence of *T. chilonis* reared on eri silkworm eggs and rice moth eggs

Generations	No-choice test		Dual-choice test	
	Eri	***Corcyra***	**Eri**	***Corcyra***
F_0	57.12±0.87a	63.73±1.23b	55.94±0.54a	61.90±0.23b
F_1	59.83±0.80a	65.37±0.41b	60.60±0.48a	61.15±0.30a
F_2	64.06±0.32a	61.82±0.55a	62.05±0.57a	60.26±0.37a
F_3	64.57±0.81b	55.61±0.71a	63.36±0.53b	56.64±0.38a
F_4	67.68±0.64b	51.76±1.60a	64.95±0.35b	54.60±0.53a
SEm+	0.29	0.27	0.50	0.37

Values are Means±SE

Means in a row followed by different letters are significantly different between two different hosts (Student's t-test, $p<0.05$).

Under dual-choice test, the percentage of female emergences displayed a comparable host-based divergence. The female percentage of *Corcyra* was higher in F_0 (61.90±0.23 percent), but Eri overtook it in F_4 (64.95±0.35 percent), demonstrating host conditioning over multiple generations. Interestingly, the proportion of female emergence in *Corcyra* declined with each generation.

The production of female offspring is a crucial measure of reproductive fitness in parasitoids, and it has a direct impact on field effectiveness and population stability (Smith, 1996) [16]. In keeping with its long-standing function in mass rearing, *C. cephalonica* initially produced a larger proportion of females in F_0. Nonetheless, the proportion of females from P. ricini rose dramatically over successive generations, surpassing *C. cephalonica* by F_3.

This change indicates better assimilation of resources and physiological compatibility over time. Li *et al.* (2018) [10] state that sex allocation strategies in parasitoids can be influenced by the internal nutritional quality and the chemical and structural features of the host chorion. The higher allocation towards female offspring may have resulted from superior protein content and internal space of *P. ricini* eggs, supporting research by Desneux *et al*. (2007) [3] on sublethal environmental factors and their effects on parasitoid reproduction.

Given that a higher female ratio guarantees sustained parasitism in field releases, this observation is crucial for the creation of mass-rearing programs (Kenis *et al*., 2023) [8].

3.4 Adult longevity under No-Choice and Dual-choice Test:

Test: Parasitoids that developed in Eri eggs had longer adult lifespans in all generations. The lifespan of adults from Eri hosts was 7.00±0.31 days in F_0 and 8.20±0.20 days in F_4. The average lifespan from *Corcyra*, on the other hand, stayed constant at 5.00-5.80 days, indicating little nutritional or developmental support under no-choice tests. Under dual-choice test, parasitoids of Eri origin outlived those of *Corcyra* origin in every generation. While F_4 parasitoids from *Corcyra* lived only 5.00±0.31 days, those from Eri hosts lived 8.60±0.24 days (Table 4.1.4). The difference in longevity was statistically significant and consistent ($t = 13.88$, $p<0.05$).

4.1.4: Adult longevity of *T. chilonis* reared on eri silkworm eggs and rice moth eggs

Generations	No-choice test		Dual-choice test	
	Eri	*Corcyra*	Eri	*Corcyra*
F_0	7.00±0.31b	5.80±0.37a	07.40±0.24b	05.60±0.24a
F_1	7.60±0.40b	5.60±0.40a	07.80±0.20b	05.60±0.24a
F_2	8.00±0.31b	5.20±0.20a	08.20±0.20b	05.40±0.24a
F_3	8.20±0.20b	5.20±0.20a	08.20±0.37b	05.00±0.00a
F_4	8.20±0.20b	5.00±0.00a	08.60±0.24b	05.00±0.31a
SEm+	0.72	1.00	0.26	0.23

Values are Means±SE

Means in a row followed by different letters are significantly different between two different hosts (Student's t-test, $p<0.05$).

In every generation and test type, *T. chilonis* adults who were multiplied on *P. ricini* eggs consistently lived longer. Because the larger host provided better nutritional provisioning during larval development, adults from Eri eggs lived noticeably longer than those from *C. cephalonica*. When multiplied on enriched host substrates or in conjunction with microbial symbionts, *T. japonicum* exhibited longer adult life, according to similar patterns reported by Wang *et al*. (2020) [20].

In parasitoids, longevity influences field persistence and searching effectiveness in addition to reproductive output. The longer lifespan seen in parasitoids raised in Eri may improve field efficacy, especially in environments with fluctuating conditions. Therefore, adopting *P. ricini* eggs in mass multiplication protocols has an additional benefit thanks to this parameter.

4. Conclusion

The results of this study unequivocally show that, in both no-choice and dual-choice scenarios, *Trichogramma chilonis* uses the eggs of *Philosamia ricini* (Eri silkworms) as a biologically appropriate and behaviourally preferred host over the course of multiple generations. Repeated exposure and selection on Eri eggs led to notable improvements in parasitisation rates, adult emergence, female progeny production, and adult longevity, even though *Corcyra cephalonica* is still a historically preferred host for mass rearing. From a sericulture standpoint, Eri eggs which are frequently left over or as by-products of commercial rearing can be used as factitious hosts for *Trichogramma* mass multiplication, which will cut down on waste and increase revenue streams for sericulture farmers. Models of sustainable agriculture and integrated rural development are well suited to this dual utility. Furthermore, the better performance of *T. chilonis* on Eri eggs suggests that these hosts may be used for both laboratory and field-level biological control program efficacy. After being reared on this alternate host, the ability of *T. chilonis* to adapt to Eri over generations highlights its potential as a potent biocontrol agent against lepidopteran pests.

In general, the incorporation of *P. ricini* eggs into biocontrol rearing systems supports sustainable agriculture and rural livelihoods by encouraging environmentally friendly pest management and boosting the sericulture industry's economic value.

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