In vitro mass reproduction of parasitic entomophages (*Braconidae, Trichogrammatidae*)

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Abstract. The issue of propagating parasitic insects in artificial food environments has been a challenge for researchers for a long time. Breeding one Trichogramma insect requires a lot of grain products and labor, and it takes about 40-50 days for Trichogramma and 55-60 days for Bracon to reproduce. This study aimed to develop a technology for the in vitro reproduction of trichogram, which are important parasitic entomophages in the control of various agricultural pests. The results of the experiments showed that the Trichogramma chilonis damage to nests with offspring was 82.4%, larvae emerged from eggs in 1.7 days. The larvae developed in this nutrient medium for 5.0 days, and the pupation period was 4.5 days. The average time from egg to imago was 11.2 days. The survival of hatchlings from the feeding medium lasted 5.4 days, and the male:female ratio (♂:♀) of hatched Trichograms was 1:6.

1 Introduction

Numerous attempts have been made by researchers worldwide to propagate parasitic entomophages in vitro, but not all have been successful. For instance, A. Donald managed to propagate Trichogramma minutum Riley for up to 10 generations in vitro, but only a few of its biological indicators were determined, including a decrease in female breeds and an increase in male breeds [1-4].

Professor Li Liying from the Guangzhou Entomological Research Institute in China successfully bred several species of Trichogramma in artificial food environments. However, the main component of his artificial food environment was the hemolymph of the oak tree moth collected from the forest experimental areas of the Guangzhou Institute of Entomology, which is a seasonal pest. This presents a problem in biolaboratories where it is not possible to breed parasitic entomophages in artificial food environments all year round [4-7].

In reality, the propagation of parasitic entomophages in artificial food environments is a pressing issue that has troubled researchers for years. The breeding of a single Trichogramma requires a significant amount of grain products and labor, taking around 40-50 days for Trichogramma and 55-60 days for Bracon to reproduce [7-11]. Biolaboratories must begin their work 90-95 days in advance to increase the mass of parasitic entomophages and carry out effective biological control against moths during the season.

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As a result, the cost of their reproduction increases, and it becomes more challenging to combat Lepidoptera pests during the season.

Efforts have been made to create artificial nutrient media for the in vitro reproduction of Braconidae, but further improvements are needed to increase efficiency. In order to address the challenge of breeding parasitic entomophages in biolaboratories, research has focused on developing methods for mass reproduction of Trichogrammatidae and Braconidae family members [4-7, 9]. This is particularly important as maintaining the necessary host species for a prolonged period of time in large quantities can be difficult. The production, storage, and isolation of effective host species in artificial nutrient media can help solve this problem. In vitro propagation of parasitic entomophages is a promising solution to these challenges, and has the potential to improve biological efficiency in controlling harmful insects [5-8, 11]. The goal of our research is to develop a technology for in vitro mass reproduction of Trichogrammatidae and Braconidae family members in biolaboratories, with a focus on creating artificial food components for their development. Our aim is to mass-produce three species of Trichogramma and two species of Braconia, which are the main parasites of the Lepidoptera order of agricultural crops, in artificial food environments [6-8, 10]. Our results demonstrate that the new method can save 2-3 times the cost, labor, and time compared to the old method, making it possible to produce a large number of parasitic entomophages in a short period of time.

2 Materials and methods

In this study, we aimed to develop a technology for the in vitro reproduction of trichogram, which are important parasitic entomophages in the control of various agricultural pests. We collected three species of trichogram (Trichogramma chilones, Trichogramma pintoi, and Trichogramma evenecens) and identified their prevalence in different districts of Tashkent region [4-7, 11]. We also collected eggs of various agricultural pests, including cabbage moth, corn moth, apple borer, bollworm, and autumn nightshade, for use in the in vitro reproduction of trichogram [6-8, 10].

To facilitate the in vitro reproduction of trichogram, we recommended two types of artificial egg cards: bag-shaped and ring-shaped. Of these, bag-shaped artificial egg cards were found to be more effective and suitable for use in the industrial production of trichogram in biolaboratories [3,4]. The bag-shaped artificial egg cards were made of plastic films and are expected to be widely used in the future for mass production of trichogram in biofactories.

To achieve the full biological efficiency of trichogram in vitro, it is important to carefully select appropriate artificial nutrient media [6-8, 9]. Further research is needed to better understand this method and to develop it for industrial-scale production of trichogram.

3 Results and discussion

Over a period of 7 years, researchers conducted over a thousand repetitions of experiments to identify the most effective nutrient medium for the efficient reproduction of members of the Trichogrammatidae family in vitro. The primary food component for the reproduction of Trichogramma species in artificial environments was their own hemolymph. Therefore, a series of experiments were conducted to effectively reproduce Trichogramma species in vitro using the hemolymphs of various insects, including the large wax moth (Galleria mellonella N), bollworm (Heliothis armigera Hb), cabbage moth (Plutella maculipenins

Curt.), cabbage white butterfly (Pieris brassicae L.), and mulberry silkworm (Bombyx mori).

The research aimed to develop convenient and effective artificial nutrient media for the future industrialized mechanism of in vitro propagation of Trichogramma species. The first stage of the research involved the preparation of artificial food media for Trichogramma species using the hemolymph of the large wax moth (Galleria mellonella N). The artificial nutrient medium was prepared using 35.0% wax moth hemolymph, 15.0% inorganic salt, 25.0% egg yolk, and 25.0% natural milk.

Contamination of the artificial food media with Trichogramma was carried out under favorable conditions for the development of Trichogramma and placed in a thermostat under these conditions. Experiments were conducted at an air temperature of +29±1ºC and a relative air humidity of 65±3.5%. The results of the experiments showed that the Trichogramma chilonis damage to nests with offspring was 82.4%, larvae emerged from eggs in 1.7 days. The larvae developed in this nutrient medium for 5.0 days, and the pupation period was 4.5 days. The average time from egg to imago was 11.2 days. The survival of hatchlings from the feeding medium lasted 5.4 days, and the male:female ratio (♂:♀) of hatched Trichograms was 1:6. (Table 1).

Table 1. Determining the hemolymph rate of wax moth in the development of Trichogramma species in artificial food environments

#	Consumption rates of the components of the artificial food medium, %				Infestation rate with Trichogramma species, %	The viability of Trichogramma offspring of different developmental periods, by days				Gender ratio (♂: ♀)
						Egg	Larva	Puparia	Imago	
1	*Trichogramma chilones*				82.4	1.7 ± 0.04	5.0 ± 0.07	4.5 ± 0.05	5.7 ± 0.05	1:6
	35 ± 0.03	15 ± 0.07	25 ± 0.04	25 ± 0.03						
2	*Trichogramma pintoe*				79.8	1.5 ± 0.02	4.4 ± 0.05	4.5 ± 0.09	4.1 ± 0.07	1:5
	35 ± 0.03	15 ± 0.07	25 ± 0.04	25 ± 0.03						
3	*Trichorgamma evenecens*				64.3	1.6 ± 0.02	4.4 ± 0.07	4.1 ± 0.05	3.7 ± 0.09	1:4
	35 ± 0.03	15 ± 0.07	25 ± 0.04	25 ± 0.03						
H (Control) wax moth egg					91.3	1.4	4.1	3.7	7.5	1:7

Trichogramma pintoi was tested in the next nutrient medium, where the level of infection with its offspring was found to be 79.8%. The average duration from egg to imago was 10.4 days, while it took 1.5 days for the larvae to emerge from the eggs. The larvae remained in the nutrient medium for 4.4 days and transformed into puparia. The pupal period lasted for 4.5 days, and the male to female (♂: ♀) sex ratio was 1:5. The experimentation for Trichorgamma evenecens species with the nutrient medium was conducted at an air temperature of +26±3ºC and a relative air humidity of 72±2.5%. The level of infection with Trichorgamma evenecens offspring in the third food medium was found to be 64.3% of the total prepared food medium, and the average duration from eggs to imago was 10.1 days. The average survival of the imagos was 3.7 days, and it took 1.6 days for the larvae to hatch from the eggs (Fig. 1). The larval period lasted for 4.4 days, and the pupal period was 4.1 days.

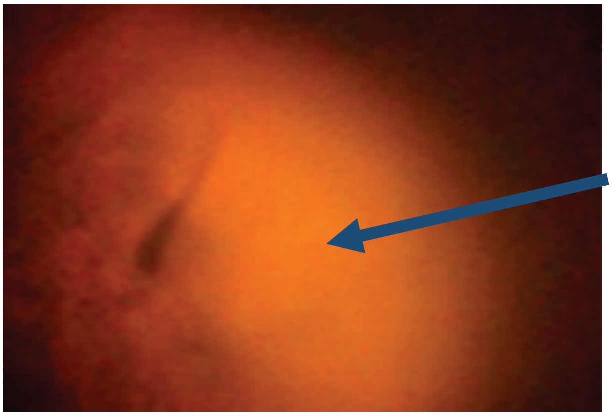

Fig. 1. Artificial eggs of *Trichogramma pintoi* the egg inside the card.

The male-to-female ratio (♂: ♀) was 1:4. A control group was used to compare Trichogramma progeny in all nutrient mediums with Trichogramma eggs under natural conditions, in which cotton bollworm and wax moth eggs were also infected with Trichogramma. The results showed that 90.5% of the control wax moth eggs were infected with trichograms, and the imagos lived for 7.5 days on average. The artificial food composition with a higher proportion of wax moth and mulberry silkworm hemolymph was found to be more effective than host hemolymph, as Trichogramma species had higher fertility, better development, and a higher male-to-female ratio (Fig. 2). The pollination of Trichogramma species was also found to be relatively different in the different nutrient mediums. These findings suggest that the artificial nutrient mediums with higher contribution of wax moth and mulberry silkworm hemolymph are more effective for the in vitro propagation of Trichogramma species than host hemolymph.

Fig. 2. Artificial eggs of *Trichogramma chilonis* offspring descendants of several stages inside the card

The development of Trichogramma chilonis, Trichogramma pintoi, and Trichogramma evenecens is known to occur in the eggs of roundworms, which are commonly infested by these species. These Trichogramma species have been found to thrive well in the hemolymph of roundworms that feed on leguminous plants, such as corn and cotton bolls, which are rich in protein and fat. Therefore, the development of these Trichogramma species is considered favorable in such conditions.

The Braconidae family, which plays a crucial role in biological plant protection, has been extensively studied. These parasites can achieve a biological efficiency of over 80% in controlling pest species of the Lepidoptera family. However, there are challenges in mass reproducing Braconidae representatives in laboratory settings. To breed Bracon, a wax moth must first be cultivated using various food items, such as wheat flour, corn, sugar, margarine, milk, and honey products. This process is labor-intensive, and it takes 55-60 days to reproduce a single generation of Braconidae representatives.

Artificial nutrient media were developed for the in vitro propagation of species belonging to the Lepidoptera family and representatives of the Braconidae family. To isolate the host hemolymph that is efficient for the development of the bracon parasite, various host hemolymphs were utilized. The hemolymphs of Galleria mellonella N (large wax moth), Heliothis armigera Hb (bollworm), Plutella maculipenins Curt. (cabbage moth), and Bombyx mori (mulberry silkworm) were studied to determine their effectiveness for the development of bracon offspring.

The research was divided into two options, A and B, to investigate the effectiveness of these pests' hemolymph for the development of bracon offspring. In addition, methods were developed to create artificial worms for the bracon in vitro. Artificial food media for bracon species were prepared using the hemolymph of each of the above-mentioned insects, and their effectiveness was studied. The main objective of the research was to identify the most effective type of insect hemolymph and develop artificial nutrient media for the future industrialized mechanism of in vitro propagation of bracon species (Fig. 3)

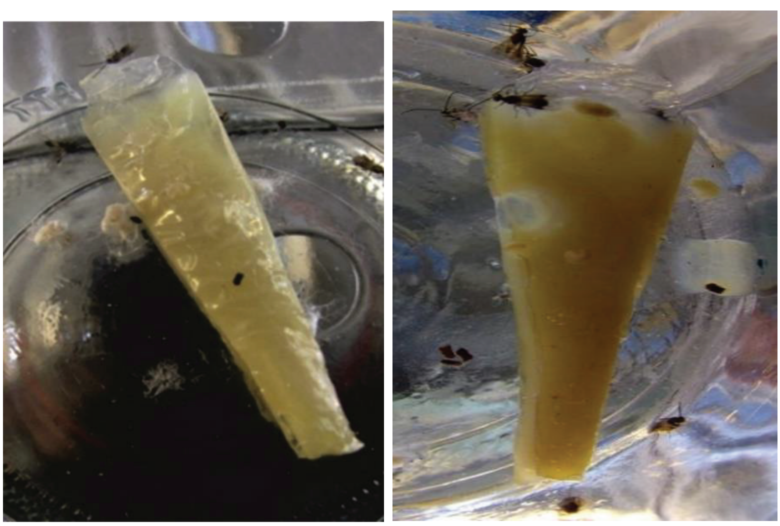

Fig. 3. The artificial worm of Tangaganotli and the bracon generations developing in it.

In the first stage of research (option A), the hemolymph of Galleria mellonella N was used in the preparation of artificial food environments for Bracon species. For Bracon hebetor, the artificial food media contained 52.0% wax moth hemolymph, 30.0% egg yolk, and 18.0% natural milk. For Bracon juglandis, the artificial food media contained 55.0% wax moth hemolymph, 22.5% egg yolk, and 22.5% natural milk, as reported by previous studies.

The infestation of artificial worms with bracon was conducted under controlled conditions of temperature and humidity using a thermostat and various microscopes such as MBS-2, MBI-3, and electron microscope Telsa-BS-613 (Czech.), as well as a MEMMERT E05273 thermostat. The thermostat was set to maintain a temperature of 30°C and a humidity level of 70% which are favorable for the development of bracon.

In the first experimental option, the infestation of artificial worms with Bracon hebetor resulted in a high success rate of 85.8% with larvae hatching observed in just 2.2 days. The larvae developed for 5.1 days in the nutrient medium and then entered the pupation stage for 4.4 days. The average time from egg to adult was 11.7 days. The hatchling survival rate in the feeding environment lasted for 8.5 days, with a male to female sex ratio of 6:21 (♂:♀) as shown in Table 2.

Table 2. Determining the rate and effectiveness of wax moth hemolymph in the development of bracken species in artificial food environments.

#	Consumption rates of the components of the artificial food medium, %			Infestation with poached offspring level, %	The viability of Bracon offspring at different stages of development, by days				Gender ratio (♂ : ♀)
					Egg	Larva	Puparia	Imago	
1	*Bracon hebetor*			85.8	2.2 ± 0.03	5.1 ± 0.09	4.4 ± 0.04	8.5	6:24
	52 ± 0.05	30 ± 0.08	18 ± 0.03						
2	*Bracon juglandis*			81.2	1.8 ±0.05	5.9 ±0.07	5.7 ±0.09	6.9	9:20
	55 ± 0.05	22.5 ± 0.07	22.5 ± 0.08						
H (Control)				95.3	1.9	5.5	4.8	8.1	1:6
Wax propeller worms									

Studies were conducted on Bracon juglandis species to investigate its infestation level in artificial worms. The results showed that the level of infestation was 81.2%, and the larvae took 1.8 days to emerge from the eggs. The larvae survived up to 5.9 days in the nutrient medium and underwent pupation for 5.7 days. The male to female ratio was 9:20, and the average time from egg to imago was 13.4 days. To compare the generation of Bracon in artificial worms with that of nightworms under natural conditions, wax propeller worms were also infected with Bracon as a control. The results showed that 95.3% of wax moths in the control were infected by poachers.

To breed Bracon hebetor and Bracon juglandis, hookworm hemolymph (45.0%), egg yolk (27.5%), and natural milk (27.5%) were used as the nutrient medium. The development of poachers in artificial food environments was carried out at a suitable temperature and humidity for the parasites. The study was conducted at a constant temperature of 30°C and 70% humidity using a thermostat. The infestation rate of artificial worms with Bracon hebetor generations was 89.2%, and the larvae hatched in 2.5 days. The larvae survived for 5.5 days in the nutrient medium, and the pupation period was 5.1 days. The average time from egg to imago was 13.1 days, and the survival of the hatchlings was

7.3 days. The male to female ratio of hatchlings was 7:19. These findings provide important insights into the development of poachers in artificial food environments.

Our research focused on the Bracon juglandis species and investigated its infestation level in artificial worms. The results showed that the level of infestation was 82.5%, and the larvae took 2.4 days to emerge from the eggs. The larvae survived for 5.6 days in the nutrient medium and underwent pupation for 5.8 days. The male to female ratio was 10:18, and the average time from egg to imago was 13.8 days. To compare the bracon generation in artificial worms with that of nightworms in natural conditions, 4-5-year-old bollworm worms were infected with bracon as a control. The results showed that 91.5% of control wax moths were infected by poachers, and the male to female ratio was 1:7 according to Table 3.

These findings provide valuable insights into the infestation level and development of Bracon juglandis in artificial worms and their comparison with nightworms in natural conditions. The study highlights the importance of understanding the impact of environmental factors on the development of poachers in different host organisms.

Table 3. Determining the consumption rate and efficiency of the hemolymph of the hookworm in the development of bracken species in artificial food environments.

#	Consumption rates of the components of the artificial food medium, %			Infestation rate with poached offspring, %	The viability of Bracon offspring at different stages of development, by days				Gender ratio (♂:♀)
					T egg	Larva	Puparia	Imago	
1	***Bracon hebetor***								
	45 ± 0.05	27.5 ± 0.07	27.5 ± 0.08	89.2	2.5 ± 0.03	5.5 ± 0.09	5.1 ± 0.04	7.3	7:19
2	***Bracon juglandis***								
	45 ± 0.05	27.5 ± 0.07	2 7.5 ± 0.08	82.5	2.4 ±0.05	5.6 ±0.07	5.8 ±0.09	5.1	10:18
H (Control) bollworm worms				91.5	1.5	4.8	4.2	8.6	1:7

In this variant, both types of Bracon were observed to infect artificial worms, but their development stages were longer than those of Bracon progeny propagated in wax moth hemolymph. Additionally, the male to female sex ratio was found to be slightly higher in these cases. The primary reason for this was identified as the artificial food medium containing less than 50% hemolymph content. Our research suggests that hemolymph should be the main component for in vitro reproduction of Bracon. To further advance the industrialized reproduction of parasitic entomophages, it may be possible to create special rooms that maintain a suitable temperature and humidity for these parasites. These findings highlight the importance of understanding the nutritional requirements of Bracon during in vitro reproduction, and could pave the way for more efficient and sustainable mass production of these important parasitic organisms.

4 Conclusions

In order to breed Trichogramma species, various artificial food media compositions were tested and it was found that those with a higher contribution of wax moth and mulberry

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silkworm hemolymph were more effective for the development and male to female ratio of the species, as well as their pollination compared to host hemolymph. These Trichogramma species were cultured on artificial egg cards in the form of a “bag”, and different artificial food environments were prepared for the effective development of each species of the Trichogrammatidae family (Trichogramma chilones, Trichogramma pintoe, Trichogramma evenecens).

For the reproduction of species of the Braconidae family that are effective in controlling Lepidoptera pests, various components of artificial nutrient media were prepared using the hemolymphs of wax moth, bollworm, cabbage moth, and mulberry silkworm.

Finally, the technology of mass reproduction of parasitic entomophages in vitro was scientifically substantiated, which has the potential to contribute to the gross reproduction of these species in the future and the industrialization of biological plant protection in the Republic.

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