

In Vitro Rearing of Parasitoids (Hymenoptera: *Trichogrammatidae* and *Braconidae*)

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Abstract: Since 2016, the studies on in vitro rearing *Braconidae* and *Trichogrammatidae* (*B. habetor* Say, *B. greeni* and *T. pintoe*, *T. evanescens*, *T. chilonis*) have been conducted successfully in Uzbekistan. For these parasites, cooking and the three main types of media were made from the hemolymph of the wax moth (*G. mellonella*) and cotton worm *Helicoverpa armigera* (*Heliothis armigera*), egg yolk, natural milk 10%. The simulated "host-eggs" and "larvae" are made of polyethylene or polypropylene semi spherical capsules, containing artificial diets, in which insectan pupal hemolymph is the main component. Mass production of in vitro reared *Braconidae* and *Trichogrammatidae* its utilization in the fields showed good effectiveness in controlling cotton bollworm.

Key words: Artificial media, parasitoid growth factors, in vitro, hemolymph, egg yolk, inorganic salt mixture, cow milk, *trichogramma*, *bracon*, predator, research tool, mass production, artificial larvae, artificial eggs, biocontrol, results.

Introduction

As the main procedure in biological control of agricultural insect, mass production of several species of parasitoids has been conducted in Uzbekistan. Their factitious hosts for mass production are still the eggs or larvae of insects. For instance, the eggs of *Helicoverpa armigera* and *Agrotis Segetum* are used for mass rearing *Trichogramma* spp. The larvae of *Helicoverpa armigera* is the factitious host for mass producing *Bracon habetor* Say or *Bracon greeni*. However, searching for more convenient and cheaper methods of parasitoid' mass rearing is still an important challenge in biological control. Manufacture of the simulated "host eggs" and "larvae" for *in vitro* rearing these parasitoids is developed rapidly too. In this paper, artificial diets and simulated hosts of these parasitoids are described[1;2;6;7].

Materials And Methods

1) Artificial diets: For *Trichogrammatidae*.

Best 3 species *Trichogramma* were tested: *T. pintoe*, *T. evanescens*, *T. chilonis*. The first 2 species are indigenous in Uzbekistan, the others were introduced from China[1;2;3;4].

This *Trichogramma* were collected from Tashkent province, Buka district, and reared in the laboratory on *Galleria mellonella*.

a) Pupa hemolymph of *Galleria mellonella* (heated in a water bath at 60 °C for 4 or 5 min) 45.5±1.5 %, chicken yolk 22.5±1.0.3 %, milk 10% / 20.5±0.5 %, inorganic salts 11.5±1.0.3 % (Neisenheimer's mixture salt NaCl7.5 g, KCl0.1 g, CaCl₂0.2 G, NaHCO₃0.2 g, H₂O 100 ml), Penicillin 400/ml. Streptomycin 400 units/ml.

b) Pupa hemolymph of *Helicoverpa armigera* 42±1.0.2 %, chicken yolk 25±1.0.8 %, milk 10% / 22.5±2.0.2 %, inorganic salts 10.5 ±0.5 %, Penicillin 400/ml. Streptomycin 400 units/ml.(Fig-1;2).

c) Pupa hemolymph of *Agretus segetum* 43±2.0.2 %, chicken yolk 24±1.0.8 %, milk 10% / 21.5±0.6 %, inorganic salts 11.5±1.0 %, Penicillin 400/ml. Streptomycin 400 units/ml. The development of *T. chilonis*, reared *in vitro* successively for many generation, is showed in Table 1[1;2;3].

Table 1.

Development of *Trichogramma chilonis*, reared *in vitro* successively for 10 generation (R.A. Jumaev. 2016 – 2017 y)

Generation	1-5	6	7	8	9	10
% parasitism	100	100	100	100	100	100
% adult emergence	89±3	88±3	87±4	86±4	86±3	85±3
% adult with expanded wings	82±3	82±2	81±3	81±2	80±3	80±2
% pupa development	93±3	92±4	92±3	90±4	90±2	87±3
% normal adult	86±2	86±3	86±4	85±2	82±2	80±3
♂: ♀ genders proportion	2:8	2:8	2.7	2.6	2:5	2:4

2) **Artificial diets for *Braconidae*:** Best 2 species *Braconinae* were tested: *Bracon hebetor* Say, *Bracon greeni*. This *Bracon* were collected from Tashkent province, Buka district, and reared in the laboratory on *Galleria mellonella* and *Helicoverpa armigera*[4;5;6;7].

Preparing of artificial mediums.

1. **Insect hemolymph collection:** Pupa were immersed in water bath at 60°C for 6 or 7 min to avoid blackening of the hemolymph. After surface sterilization with alcohol and need sterile condition.
2. **Chicken embryo extract collection:** The Chicken embryo extract, only it should be the egg yolk and need sterile condition.
3. **Milk:** Fresh cow milk or 10% powdered milk solution also need sterile condition.
4. **Inorganic mixture salt:** Use Neisenheimer’s mixture salt (NaCl7.5 g, KCl0.1 g, CaCl₂0.2 G, NaHCO₃0.2 g, H₂O 100 ml).

It is defined there is albumen, oil, water when it is checked ingredients of master caterpillar’s type that belong to *Bracon* in nature. Besides, *Heliothis armigera* Hb, *Agrotis segetum* Sciss and *Galleria mellonella*’s pupa liquid is used for research (Fig-2).

Natural milk 10% mixture is added to hemolymph and hicken yolk is added diet medium and put at ultraviolet lamp. It is rotated for 5 minutes at 2000 second speed in centrifuge. Diet medium should be kept in the clean, without microbe room and at 20°C cool [1.3.5;6;7].

After medium has been ready, it is placed in special artificial caterpillars which are cleaned with 75% ethyl spirit made of politilen.

MANUFACTURE OF SIMULATED "ARTIFICIAL EGG-CARDS AND LARVAE"

1) **Artificial “egg-cards”:** There are 2 types of artificial “egg-cards”[1;2;3].

a) Tri-ring “egg-cards” 2 pieces of plastic film are used. The semispherical concaves are made on the upper plastic film. Artificial medium is poured into concaves fully (but without overflow) with a micro-syringe or micro-pipette. The bottom plastic film has no concaves. The upper and bottom plastic films are separated and stretched tightly by three plastic ring with different inner diameters, in our Cass they are: 5:5 cm, 5.4 cm, 5.2 cm respectively.

b) Bag-form “egg-cards”:

One half of a piece of film is fulfilled of concaves with medium and is covered by another half of film. Three sides of film are sealed. Convex side of capsules are exposed and between the concave side and bottom half of the same piece of film there should be space for aeration. The size of plastic film depends upon the number of semispherical concaves made on film[1;2;3].

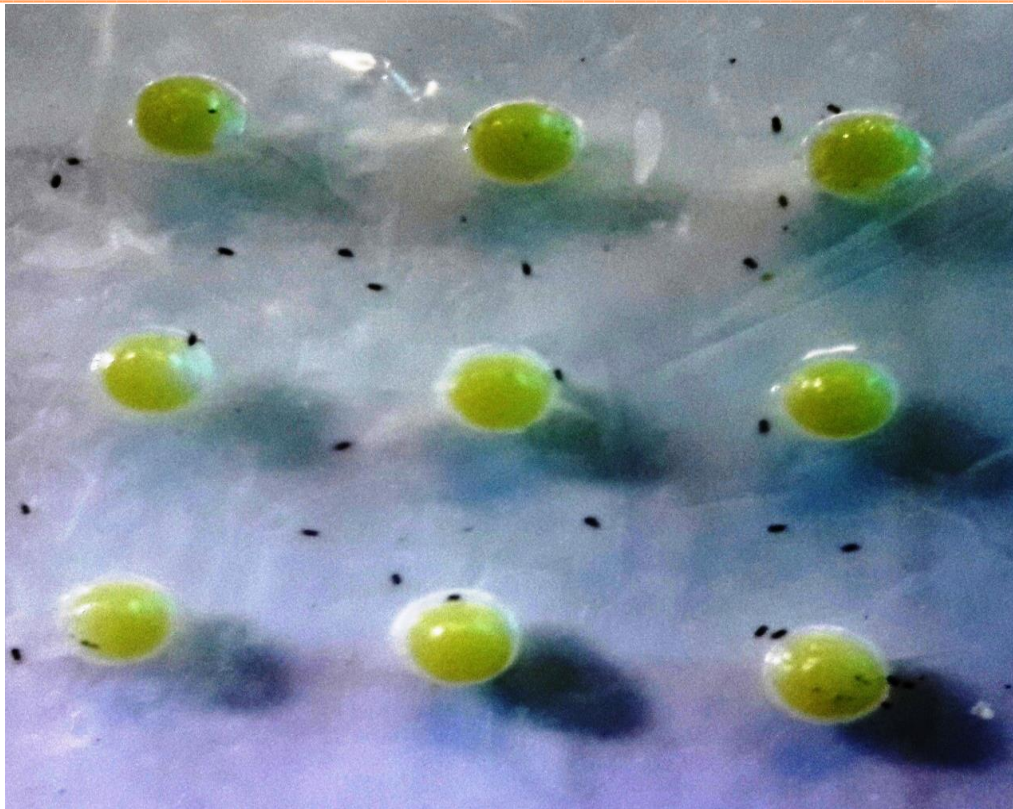


Fig-1. Bag-form artificial “egg - card”

2) Produce depicted artificial larvae: A small part (1x1) of parafilm is extended 2x4 cm, absorbed in 75% alcohol for 15 minutes. Then it is dried with sterile printed paper, folded as a sack and fixed in order to stick both sides. 0,5 ml of mediums is placed by pipette into each parafilm box.

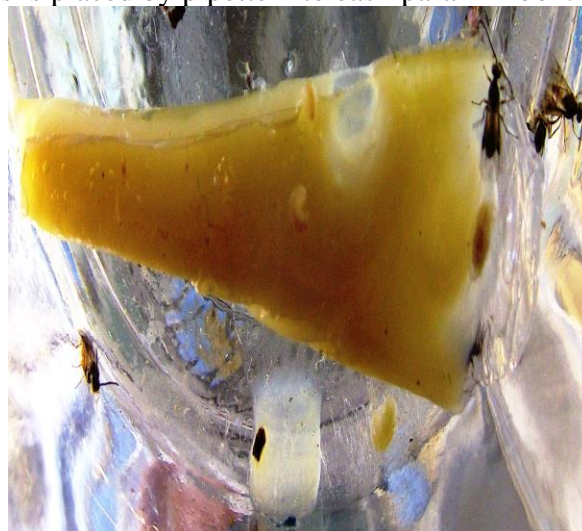


Fig-2. Artificial larvae

When parafilm box is filled with mediums, it yields such depicted maggot. 15-20 small holes are opened with sterile entomologic needle for properly prepared artificial caterpillars. All the processes of the preparation are required to carry out in sterile room [1;3;4;5;7].

Results Of In Vitro Reared Parasitoids

1) It was showed in the Table 1 that the pupae hemolymph of either *G.melonnellan* or *H.armigera* Hb could be used as the main component of the artificial diet for the development of *Trichogrammatidae*. There was no significant difference in their parasitism, survival, percentage of pupation, adult emergence and reproductively when the pupae hemolymph of *G.melonnella* was used instead of that of *H.armigera* Hb.



Fig-3. a) Artificial larvae and pupa

It is obvious in the research, prepared all mediums of diets are harmed with *Trichogrammatidae* generation and put there eggs. But it is observed dying because of inconvenience medium of diets for developing parasite generation. According to diet mediums component development of *Trichogrammatidae* parasite was rather prolonged when *G.melonnella* hemolymph and inorganic salt's quantity was more in diets medium. In fact quantity of protein and oil is more in the structure of hemolymph as well as it is considered convenient for development of parasite maggot[1;2;3].

Thus, from above nominated artificial diet mediums, pupae hemolymph of *G.melonnella* 45.5 ± 1.5 %, chicken yolk 22.5 ± 1.03 %, milk 10% / 20.5 ± 0.5 %, inorganic salts 11.5 ± 1.03 % composed medium of diets is defined as a suitable for normal nourishment and development of *Trichogrammatidae* generation in order to rear trichogramma parasite[1;2].

2) We chose two type of *Bracon*. Bracons, *Bracon hebetor* Say and *Bracon greeni*, this type is tolerant, durable in extreme condition. It is practiced by means of choosing convenient state in developing each type to be harmed diet mediums with Bracon and in this condition it is put in thermostat(Fig-4).

Damaging the type of *Bracon* with diet mediums at $30 \pm 1^\circ\text{C}$ temperature, at $68 \pm 3\%$ moisture.

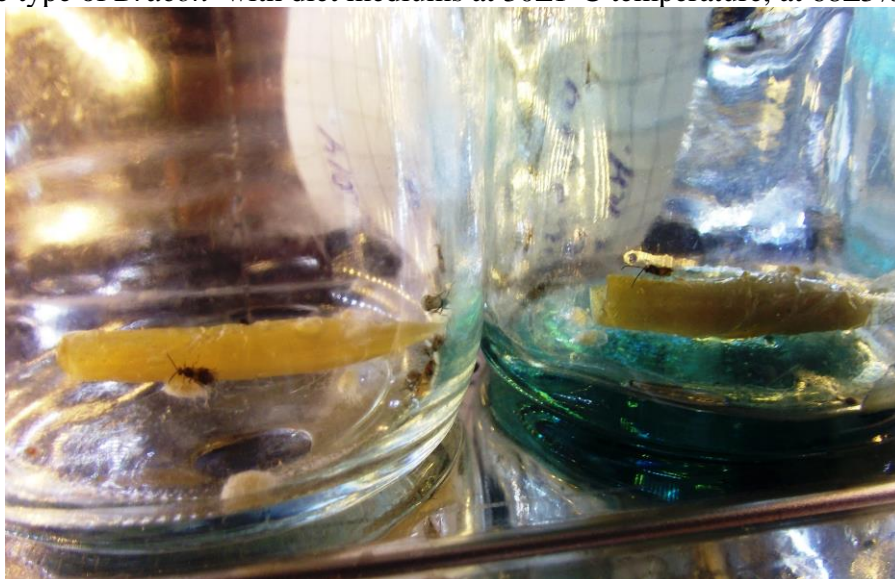


Fig-4. In vitro reared Braconidae.

Thus, in order to develop and consume of *Braconidae* generation well from above mentioned artificial diet, mediums hemolymph wax moths (*G.melonella*) 40,04%, chicken yolk 30,03%, milk 10% 29,03% is confirmed as the best diet medium to rear *Braconidae* parasite[1;5;6;7].

References

1. **R.A. Jumaev.** In vitro rearing of *Trichogramma* (Hymenoptera: Trichogrammatidae) European science review. – 2016. - № 9 – 10. - pp. 11–13. (In Vienna).
2. **Li Li-ying, Liu Wenhui.** Rearing *Trichogramma spp*, with artificial diets, containing hemolymph of different insects. Parasitoids and predators (insecta) of agricultural and forestry arthropod pests - 1997. -pp 335-337. (In china)
3. **Li Li-ying, Liu Wenhui, etc.** In vitro rearing of *Trichogramma spp* and *Anastatus sp* in artificial “eggs” and the methods of mass production. Parasitoids and predators (insecta) of agricultural and forestry arthropod pests.-1997. –pp.344-357.(In china).
4. **Кимсанбаев. Х.Х., Жумаев Р.А.** Renewing and rearing technology of *Bracon hebetor* Say in Biolaboratory; Материалы VIII-оймеждународной научно - практической конференции молодых исследователей, г. Волгоград, 2014. –pp.257-259.
5. **Huang Xin.** Study on the use of *Habrobracon hebetor*. Bulletin of Biological Control 2 (2): -1986. – pp. 70 – 75 (In Chinese).
6. **Xie Zhong-neng, etc.** In vitro culture of *Habrobracon hebetor* (Say) (Hym.,Braconidae).Chines J.of Biological Control // 1989. - № 5(2): –pp. 49 – 51. (In China).
7. **Xie Zhong-neng, etc.** In vitro culture of the ectoparasitoid *Bracon greeni* Ashmead. Acta Entomologica Sinica // 1989. - № 32(4): –pp. 433 – 437. (In China).