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TECHNOLOGY FOR IN VITRO REARING OF PARASITICS

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Abstract
The technology of in vitro propagation of parasitic entomophages is a very important process. This direction is very important for the agricultural economy. An average of 1.8-2.2 million dollars is spent on the propagation of parasitic entomophages in our country per year. If we introduce the technology of in vitro propagation of parasitic entomophages, this will reduce costs by 2-3 times. Propagating parasitic organisms in this way will save significant economic costs.

The studies on in vitro rearing Hymenoptera 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.melonnella) and cotton worm Helicoverpa armigera (Heliothis armigera), egg yolk, natural milk 10%. Mass production of in vitro reared Braconidae and Trichogrammatidae its utilization in the fields showed good effectiveness in controlling agricultural economy.

Keywords Agricultural economy, artificial media, parasitoid growth factors, in vitro, hemolymph, egg yolk, inorganic salt mixture, cow milk, 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].

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 0C 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].

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].

Table 1.

DEVELOPMENT OF HYMENOPTERA, REARED IN VITRO SUCCESSIVELY FOR 10 GENERATION

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

Preparing of artificial mediums.

1. Insect hemolymph collection: Pupa were immersed in water bath at 600C for 6 or 7 min to avoid blackening of the hemolymphy. 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 fulled 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].

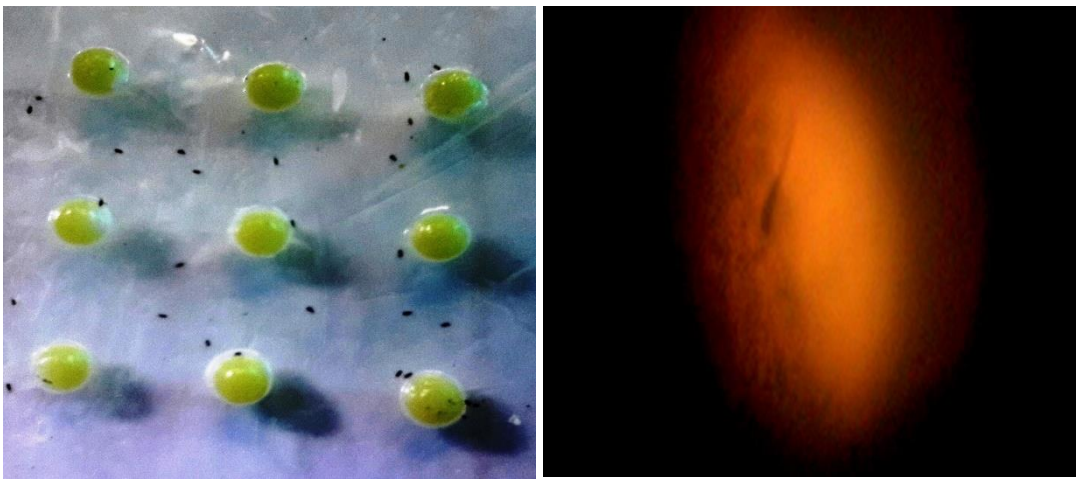

Fig-1. Bag-form artificial "egg - card" and egg.

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. a) Artificial larvae and pupa (*Trichogramma chilonis* Ishii)

When parafilm box is fulled 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

It was showed in the Table 1 that the pupae hemolymphy 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 hemolymphyof G.melonnella was used instead of that of H.armigera Hb.

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 hemolymphy and inorganic salt's quantity was more in diets medium. In fact quantity of protein and oil is more in the structure of hemolymphy as well as it is considered convenient for development of parasite maggot[1;2;3].

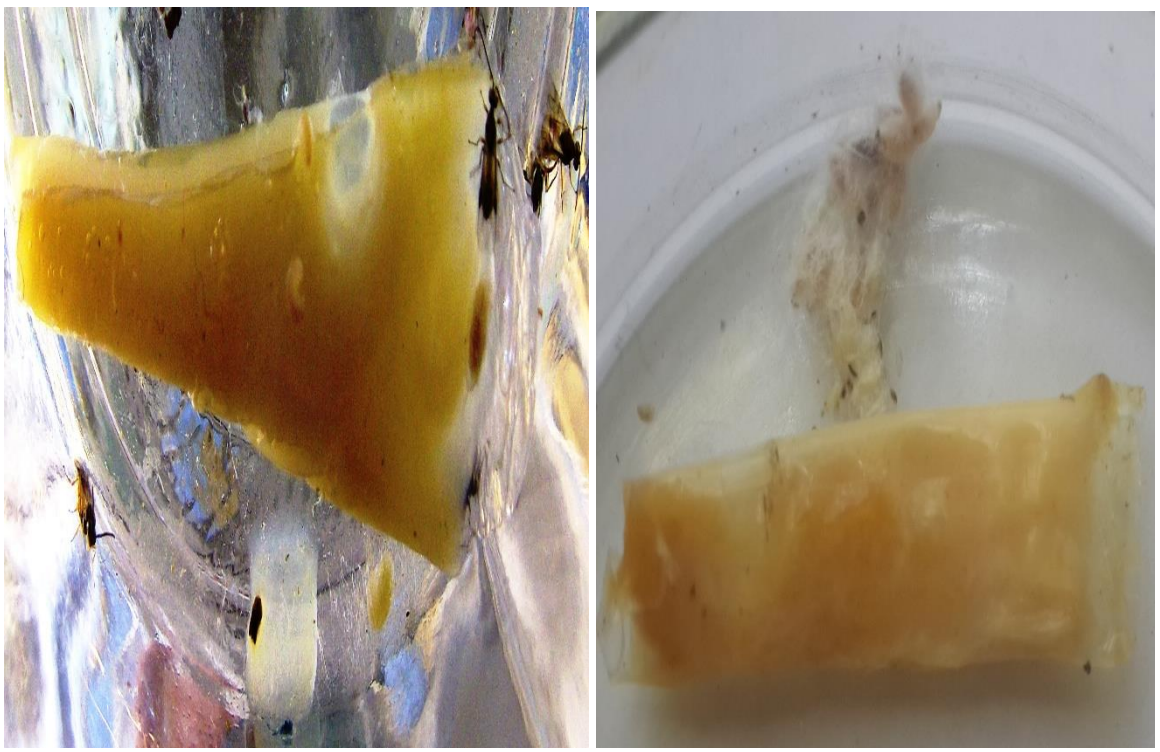

Fig-3. Artificial larvae (*Bracon hebetor* Say)

Thus, from above nominated artificial diet mediums, pupae hemolymph of G.melonnella 45.5+1.5 %, chicken yolk 22.5+1,0.3 %, milk 10% / 20.5+0.5 %, inorganic salts 11,5+1,0.3 % 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].

Fig-4. In vitro reared Braconidae.

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±1°C temperature, at 68±3% moisture.

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].

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