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BIOTECHNOLOGICAL METHOD REARING OF *TRICHOGRAMMA PINTOI*

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Abstract. *The article discusses scientific research on the biotechnological feeding of Trichogramma pintoi. In the preparation of food, a nutrient medium was prepared for biotechnological feeding based on the hemolymph of the wax moth (G.melonnellan). Wax moth (G.melonnellan) sponges are important because they are constantly in the biolaboratory. Hemolymph was isolated from the sponges and used for mass feeding of Trichogramma species by biotechnological methods. The research was devoted to the use of hemolymphs of various insects, but the best results were achieved with the hemolymphs of the mulberry silkworm (Bombyx mori) and wax moth (G.melonnellan).*

Key words: *Artificial media, parasitoid growth factors, in vitro, hemolymph, egg yolk, inorganic salt mixture, cow milk, trichogrammatidae, predator, research tool, mass production.*

Annotatsiya. *Maqolada Trichogramma pintoi ning biotexnologik oziqlanishi bo‘yicha ilmiy tadqiqotlar muhokama qilinadi. Ozuqa tayyorlashda mum parvonasi (G.melonnellan) gemolimfasi asosida biotexnologik oziqlantirish uchun ozuqa muhiti tayyorlandi. Mum kuyasi (G.melonnellan) bulutlari muhim ahamiyatga ega, chunki ular doimo biolaboratoriyada bo‘ladi. G‘ovaktanlilardan gemolimfa ajratib olindi va biotexnologik usullar yordamida trixogramma turlarini ommaviy oziqlantirishda foydalanildi. Tadqiqotlar turli hasharotlar gemolimfalaridan foydalanishga bag‘ishlangan bo‘lib, eng yaxshi natijalarga tut ipak qurti (Bombyx mori) va mum parvonasi (G.melonnellan) gemolimfalaridan foydalanilganda erishildi.*

Kalit so‘zlar: *Sun’iy muhitlar, parazitlarning o‘sish omillari, in vitro, gemolimfa, tuxum sarig‘i, noorganik tuzlar aralashmasi, sigir suti, trixogrammatidlar, yirtqich, tadqiqot quroli, ommaviy ishlab chiqarish.*

Аннотация. *В статье обсуждаются научные исследования по биотехнологическому питанию Trichogramma pintoi. При приготовлении пищи была приготовлена питательная среда для биотехнологического питания на основе гемолимфы восковой моли (G.melonnellan). Губки восковой моли (G.melonnellan) важны, потому что они постоянно находятся в биолаборатории. Гемолимфа была выделена из губок и использована для массового питания видов Trichogramma биотехнологическими методами. Исследования были посвящены использованию гемолимф различных насекомых, но наилучшие результаты были достигнуты с гемолимфами тутового шелкопряда (Bombyx mori) и восковой моли (G.melonnellan).*

Ключевые слова: *искусственные среды, паразитоидные факторы роста, in vitro, гемолимфа, желток яйца, неорганическая солевая смесь, коровье молоко, trichogrammatidae, хищник, исследовательский инструмент, массовое производство..*

At the process of entirely rearing *trichogramma* parasite in the laboratory condition cultivates *Sitotrogoce reallela* Olive egg a lot of corn productions such as grain, maize, barley and worker force are spent. In the result of it, rearing price is increased. More than 150 thousand ton barley is emitted in exist biolaboratory our Republic in a year[1.2.3.]

Emitting this barley, other diets cultivation happens in the account of decreasing producing of plants or enterprises. This testifies that above mentioned process is damaging for diets industry[1.2.].

By rearing trichogramma commonly using artificial diets mediums and automatizing them gives an opportunity to solve above problem.

In China 1975-1978 years for 3 year’ investigations rearing *trichogramma* in the diets medium, which belongs to artificial medium researches showed its good results[1.3.].

According to the first medium, first general ingredients: (Hemolymph from pupae *Antheraea pernyi* 43.1 % , egg yolk 34.48% , inorganic salt mixture 13.79 % , and pig serum 8.62 % or hemolymph from pupal *Philosamia cynthia* ricini 31.75%, cow milk 31.75%, egg yolk23.81 % and , inorganic salt mixture12.70%[2.3.].

By the method of rearing Entomophags in vitro many scientists investigated and achieved positive results

According to Gao and others’ (1982) researches trichogramma generation which is reared in vitro when is used against *H.armigers* in the field of cotton-plant was achieved biologic effectiveness by 93%[1.2.].

The artificial rearing of *trichogrammatidae* started a long time ago, with the main goal to try obtaining a mean to multiply and produce parasitoids to be released in biological control strategies. But it is also a powerful tool to conduct studies on biology, physiology and behavior of entomophags, especially endoparasitoid species (Grenier, S., Plantevin, G. 1990.). *Trichogramma* spp 5 are widely used for biological control of pest insects. Currently, these egg parasitoids can be produced only from natural or factitious hosts or from insect hemolymph-based diets [1-15].

Wu *et al.* (1982), Liu and Wu (1982), and Qin and Wu (1988) described artificial diets devoid of insect components for the *in*

vitro rearing of *Trichogramma dendrolimi* Matsumura[2.3.].

Considerable work has been done on the development of artificial diets for *Trichogramma* spp. Many of these efforts have involved diets containing hemolymph (Guan *et al.,* 1978; Hoffman *et al.,* 1975; Hubei Research Group, 1979; Liu *et al.,* 1979, 1982; Wu *et al.,* 1982; Strand and Vinson, 1985; Xie*t al.,* 1986, 1997a; Qin and Wu, 1988; Grenier*et al.,* 1995). Development of artificial diets for *Trichogramma* would foster the implementation of automated mass rearing systems. Some progress has been made along this line, primarily in China (U. S. Department of Agriculture, 1982; Li *et al.,* 1988, 1995a,b) where *in vitro* reared *Trichogramma chilonis* Ishii have been released for control of sugarcanestem borers (Liu *et al.,* 1995b) and *Trichogramma dendrolimi* Matsumura has been reared for control of *Heliothis armigera* L. in cotton and *Dendrolimus punctatus* L. in pine trees[1.3.].

Source of *Trichogramma* and In Vitro Rearing *Trichogramma* stock: *T. evenecens, T.pintoe* were collected from Tashkent province, Buka district, and reared in the laboratory on *Heliothis armigera* and *Agretus segetum* shif.

Ingredients for in vitro medium:, Pupae hemolymphy (*G.melonnellan* or *H.armigera*), cow milk or 10% powdered milk solution, chicken embryo extract and Neisenheimer’s mixture salt.

Insect hemolymph collection: Alive pupae were immersed in water bath at 60^{0}C for 6 or 7 min to avoid blackening of the hemolymphy. After surface sterilization with alcohol and need sterile condition.

Chicken embryo extract collection: The Chicken embryo extract, only it should be the egg yolk and need sterile condition.

Milk: Fresh cow milk or 10% powdered milk solution also need sterile condition.

Inorganic mixture salt: Use Neisenheimer’s mixture salt (NaCl7.5 g, KCl0.1 g, $CaCl_2$0.2 G, $NaHCO_3$0.2 g, H_2O 100 ml).

Artificial “egg-cards”

There are 2 types of artificial “egg-cards”[2.3.].

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

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 [1-15].

Culturea of *G.melonnella or H.armigera* fresh cocoons of *G.melonnellan* or *H.armigera* from Tashkent province.

Components of artificial diets for *Trichogramma pintoi*

The first medium: Hemolymph (A) G.melonnellan (A_1) 45,5 %, Neisenheimer’s (A_2) 15,5 %,Chicken egg yolk (A_3) 25,5 %, milk (A_4) 13,5 %.

The second medium: Hemolymph (B) *Heliothis armigera Hb* (B_1)34,5%, Neisenheimer’s (B_2) 12,5 %, Chicken egg yolk (B_3) 30,5 %, milk (B_4) 22,5 %.

The three medium: Hemolymph (C) *Agretus segetum shif* (C_1)42,5%, Neisenheimer’s (C_2) 13,5 %, Chicken egg yolk (C_3) 23,5 %, milk (C_4) 20,5 %.

The four medium: Hemolymph (D) *G.melonnellan* (D_1) 45,5 %, Neisenheimer’s (D_2) 12,5 %, Chicken egg yolk (D_3) 30,5 %, milk (D_4) 11,5 %.

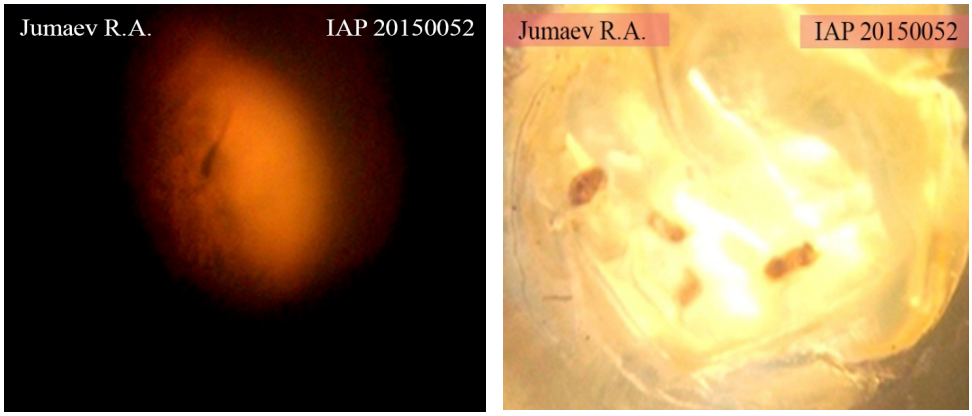
The five medium: Hemolymph (E) *G.melonnellan* (E_1)45,5 %, Neisenheimer’s (E_2) 13,5 %, Chicken egg yolk (E_3) 20,5 %, milk (E_4) 20,5 %

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 *T. pintoi and T. evenecens*. There was no significant difference in their

Table 1

Development of *Trichogramma pintoi*, reared in vitro laboratory experiences. (2024-2025 yy.), (+28±1^{0}C, RH 65±5 %)

Composition of medium, %				The amount of damage, %	Development degree of trichogramma generation in artificial medium by days				Genders proportion ♂: ♀
					Eggs	larva period	Pupa period	Mature period (imago)	
A				65,5	1,5	5,2	4,5	3,7	1:4
A_1	A_2	A_3	A_4						
45.5	15.5	25.5	13.5						
B				45,5	1,6	3.5	2,2	2,0	3:5
B_1	B_2	B_3	B_4						
34.5	12.5	30.5	22.5						
C				35,0	1,1	4,2	2.4	1,2	2:2
C_1	C_2	C_3	C_4						
42.5	13.5	23.5	20.5						
D				72,1	1,2	5,5	4,1	3,0	2:6
D_1	D_2	D_3	D_4						
45.5	12.5	30.5	11.5						



a b

1-Fig. Developmental stages of *T. evenecens* by in vitro
a-egg,
b-larvae of 2 nd instar, early stage of pre pupae and female adult

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 *trichogramma* 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 *trichogramma* 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 [1-15].

Thus, from above nominated artificial diet mediums, fifth diet medium (E) pupae hemolymph of *G.melonnella* (E_1) 45,5 %, inorganicsalts (E_2) 13,5 %, chicken yolk (E_3) 20,5 %, milk (E_4) 20,5 % composed medium of diets is defined as a suitable one for normal nourishment and development of *trichogramma* generation in order to rear *trichogramma* parasite.

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