CHEMISTRY AND CHEMICAL ENGINEERING

Volume 2019 | Number 3 Article 48

August 2019

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SELECTION OF OPTIMAL CONDITIONS FOR THE CULTIVATION OF INSECT CELLS FOR USE IN BIOTECHNOLOGY

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The article discusses use of the insect cell cultures for biotechnological purposes. Optimal conditions for the cultivation of Spodoptera exigua and Lymantria dispar cell lines were selected.

Keywords: insect cell culture, in vitro, Spodoptera exigua, Lymantria dispar, insecticidal activity, nutrient medium.

ВЫБОР ОПТИМАЛЬНЫХ УСЛОВИЙ КУЛЬТИВИРОВАНИЯ КЛЕТОК НАСЕКОМЫХ ДЛЯ ИСПОЛЬЗОВАНИЯ В БИОТЕХНОЛОГИИ

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В статье обсуждается использование культур клеток насекомых в биотехнологических целях. Подобраны оптимальные условия культивирования клеточных линий Spodoptera exigua и Lymantria dispar.

Ключевые слова: культура клеток насекомых, in vitro, Spodoptera exigua, Lymantria dispar, инсектицидная активность, питательная среда.

BIOTEXNOLOGIK USULLARDAN FOYDALANGAN HOLDA HASHAROT HUJAYRA LINIYALARINI KO'PAYTIRISHNING MAQBUL SHAROITLARINI TANLASH

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Maqola hasharot hujayra kulturasidan biotexnologik maqsadlarda foydalanishga qaratilgan. Spodoptera exigua va Lymantria dispar hasharotlarining hujayra liniyalarini laboratoriya sharoitida ko'paytirishning optimal sharoitlari aniqlangan.

Kalit so'zlar: hasharot hujayra kulturasi, in vitro, Spodoptera exigua, Lymantria dispar, insektisid faollik, ozuqa muhiti.

Kirish

Biotexnologiyaning rivojlanishida hujayralar kulturasi ustidagi amaliyotlar katta ahamiyat kasb etib, hozirgi kunda ular qo'llaniladigan sohalar yanada kengayib bormoqda. Ushbu yo'nalishlar orasidan ikki soha: hasharot hujayralari va ularning bakuloviruslaridan foydalangan holda rekombinant oqsillar olish [1-6] va qishloq xo'jaligi turli zararkunanda hasharotlariga nisbatan insektisid faollikni hujayra kulturalarida *in vitro* tadqiq etish yanada izchil rivojlanib bormoqda [7-10].

Tadqiqotning asosiy maqsadi *Spodoptera exigua (Karadrina, kichik quruqlik tunlami)* va *Lymantria dispar (Limantriya, neparno'y shelkopryad)* hasharotlari hujayra liniyalarini laboratoriya sharoitida ko'paytirishning optimal sharoitlarini aniqlashdan iboratdir. Mazkur hujayra liniyalari ushbu zararkunanda hasharotlarga nisbatan insektisid faollik ega tabiiy va sintetik birikmalarni qisqa muddatlarda, ekologik toza usulda va kam harajat sarflagan holda izlab topish imkoniyatini beradi.

Tadqiqot usullari

Tajribalar uchun_SIGMA-ALDRICh (Merck, Germaniya), New England Biolabs (SShA), Gibco (SShA), HiMedia Laboratories Pvt.Ltd (Indiya) firmalarining ferment va reagentlaridan foydalanildi.

Spodoptera exigua va *Lymantria dispar* hujayra liniyalari O'simlik moddalari kimyosi instituti Molekulyar Genetika laboratoriyasi hasharot hujayralari kollektsiyasidan olingan bo'lib, haftada bir marotaba subkultivatsiya yo'li bilan ko'paytiriladi. Hujayra liniyalarining passajlari soni 1000 tadan oshadi.

Hujayralarni o'stirish uchun ishlatiladigan ozuqa muhitlari:

Hasharot hujayra liniyasi uchun 90 % Grace's

Buzoq embrioning 10 % li zardobi

1 % fungizon (amfoteritsin V) na 250 g'ml. *Cat. No. 15290-018*.

1 % 10 000 UG'ml penitsillin G - streptomitsin 1 mg'ml. *Cat. No. 15140-148.*

Hujayra liniyalarini ko'chirib o'tkazish tartibi:

Ushbu turdagi hasharot hujayralari hafta davomida bir marotaba qayta ko'chirib o'tkazishni talab qiladi (hujayralar o'stiriluvchi flakondan). Buning uchun termostatdan optimal o'sish harorati 26 ^{o}S da turgan flakonlar chiqariladi va muzlatgichda 4 ^{o}S da 20 min davomida ushlab turiladi.

- Keyin idish foydalanilgan ozuqa muhitdan tozalanadi.

-Hujayraning yuza qismi 3 ml tripsin buferi bilan tezda yuvib tashlanadi.

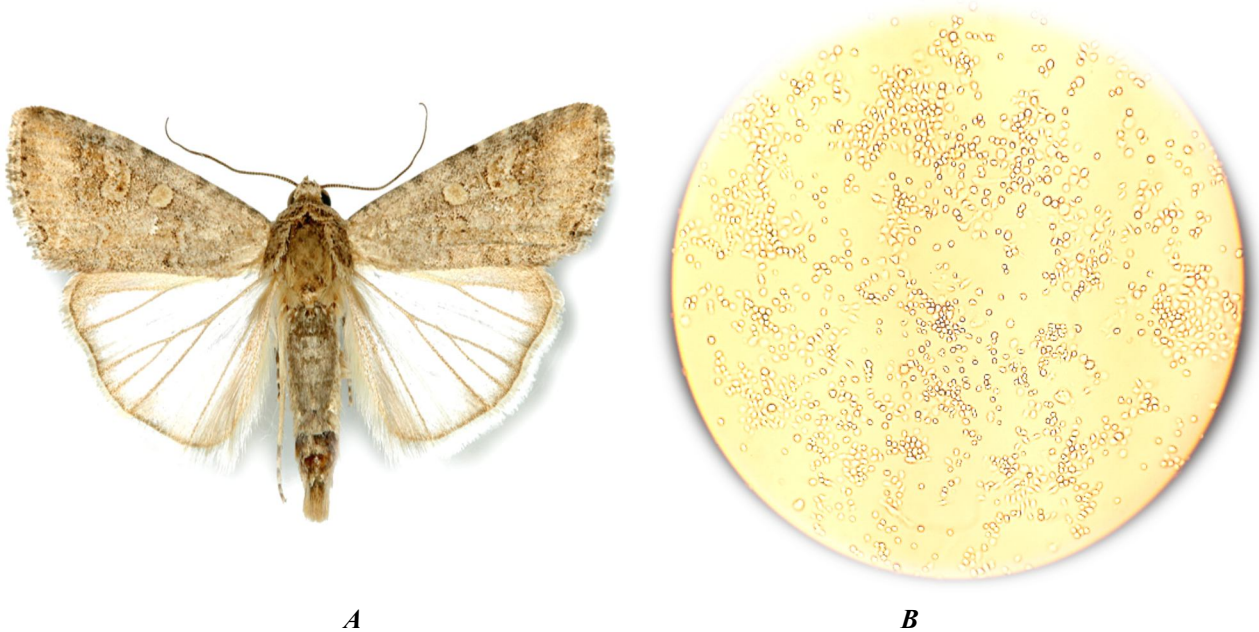
- 3 ml 0.05% li tripsin eritmasi qo'shilib, 4 °S da 5-7 minut davomida inkubatsiya qilinadi (hujayraning flakonga bog'lanish darajasiga qarab).

- Flakondan tripsin olib tashlanadi.

- 5 ml toza ozuqa muhit qo'shiladi va hujayralarni flakon devoridan zudlik bilan pipetka yordamida yuvib tushiriladi.

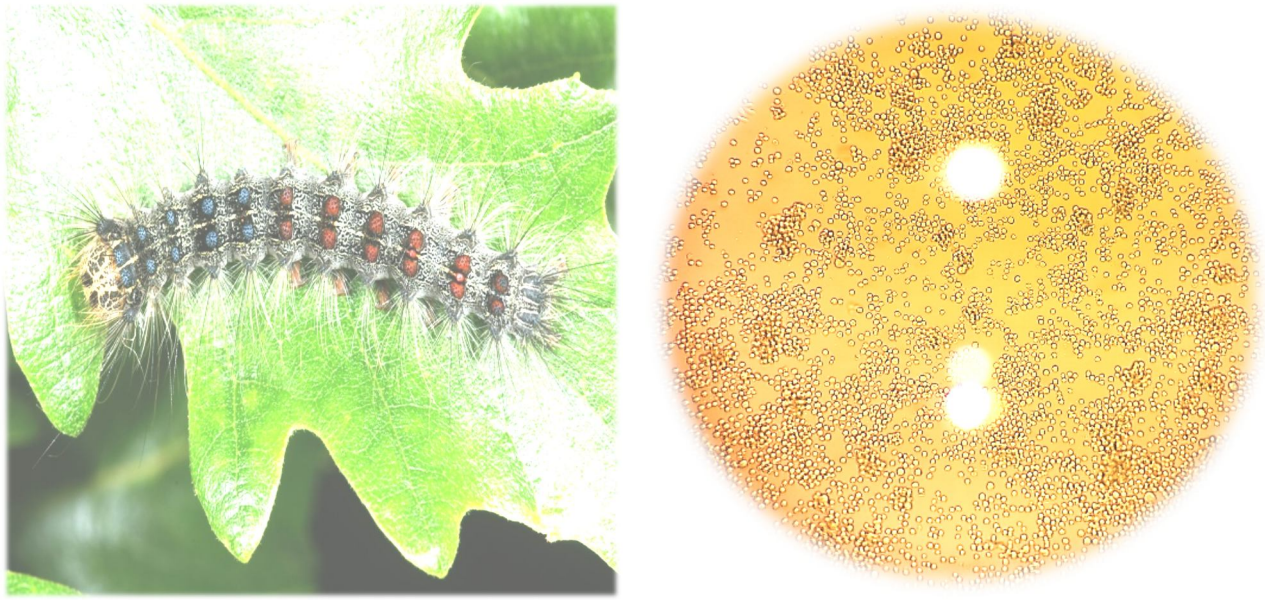
- Yangi steril idishga 3.5 ml Grace's li ozuqa muhitidan solinib, uning ustiga 0.5 ml hujayrali muhitdan qo'shiladi.

- Ekilgan yangi hasharot hujayra liniyalari



A ***B***

1-rasm. *Spodoptera exigua* hasharoti (A) va hujayralarining mikroskop ostida ko'rinishi (B).



A ***B***

2-rasm. *Lymantria dispar* lichinkasi (A) va hujayralarining mikroskop ostida ko'rinishi (B).

keyingi ekish davrigacha termostatda 26 ^{o}S da inkubatsiya qilinadi.

Tirik hujayralar sonini aniqlash:

- Buyum oynasiga bir tomchi hujayrali ozuqa muhiti bilan birga yana bir tomchi Tripan ko'k eritmasidan tomizilib aralashtiriladi. Bunda Tripan ko'k o'lik hujayralarni bo'yaydi, bu esa o'z navbatida bizga tirik hujayralar sonini aniqlash imkonini beradi.

- Buyum oynasi oyna qoplagich bilan yopiladi va mikroskopga joylashtiriladi.

- 10 x kattalashtirish 1 sm^2 joydagi tirik hujayralar sonini aniqlash uchun yetarli hisoblanadi.

- Tanlangan kvadratdagi hujayralar soni gepatotsitometrda hisoblab chiqiladi va quyidagi formula orqali ularning umumiy soni aniqlanadi:

1 ml hajmdagi tirik hujayralar soni q 1 mm^2 dagi hujayralar sonini x suyultirish x 10^4 ga ko'paytirilganiga teng bo'ladi.

Natijalar va muxokama

Ma'lumki, turli xil zararkunanda hasharotlar qishloq xo'jaligi ekinlariga jiddiy ziyon yetkazishadi. Shu jumladan, *Spodoptera exigua* va *Lymantria dispar* hasharotlari ham asosiy zararkunandalardan hisoblanadi.

Spodoptera exigua (1-rasm) - kichik quruqlik tunlami, tunlamlar oilasiga mansub kapalak. Tanasining uzunligi 11-13 mm, qanotlari yoyilganda 23-34 mm. Tuxumini (16-150 ta) to'p to'p qilib o'simlikka, kuzda esa tuproqqa qo'yadi. Yashil yoki qo'ng'ir-kulrang tusdagi qurtining uzunligi 2,5-3,0

sm. Havo sharoitiga qarab yilda 2-8 avlod beradi. Har xil iqlim mintaqalarida va yil fasliga qarab kapalagi ma'lum ozuqa o'simligini tanlaydi. Dukkakdoshlar, g'alladoshlar, sho'radoshlar, piyozguldoshlar va boshqa oilaga mansub o'simliklar bilan oziqlanadi. G'o'za, beda, tamaki, lavlagi, soya, jo'xori, tomat, kartoshka, baqlajon, qalampir va boshqa o'simliklarning xavfli zararkunandasi hisoblanadi.

Lymantria dispar (2-rasm) - polifag bo'lib, 300 ga yaqin o'simlik turi, asosan o'rmon daraxtlari va butalarga katta ziyon yetkazadi. Eman, qayin, terak, olxo'ri va olma daraxtlarining asosiy zararkunandasi hisoblanadi. Erkak va urg'ochi kapalaklari shakli va rangi jihatidan biri biridan jiddiy farq qiladi. Havo sharoitiga qarab butun yoz davomida ko'payadi.

Tadqiqotlarimizni ilmiy adabiyotlarda keltirilgan ma`lumotlardan foydalangan holda, hujayra liniyalarini 20% li embrion zardobi (Fetal Bovine Serum, HiMedia Laboratories Pvt.Ltd) saqlovchi Grace's ozuqa muhitida +18ºC harorat ostida 7 kun davomida o'stirishdan boshladik [11]. Ozuqa muhitidagi zardobning foiz kontsentratsiyasini 10% va 15% ga tushirilsa hujayralarning o'sish potentsiyali keskin pasayganligiga guvoh bo'ldik. Ozuqa muhitining xuddi shunday nisbatlarida haroratni +19ºC dan +26ºC gacha gradientli o'zgartirilishi hujayra liniyalarining ko'payishiga sezilarli ta`sir etishi aniqlandi. Tajribalar natijasiga ko'ra tarkibida 15% zardob saqlovchi ozuqa muhiti va +22ºC harorat, shuningdek 10% zardob saqlovchi ozuqa muhiti va +26ºC harorat ostida hujayra liniyalarini ko'paytirish eng optimal sharoitlar ekani ko'rsatib berildi va nisbatan qimmat turadigan FBS reagentining tejalishiga erishildi.

Xulosa

Tadqiqot natijalariga ko'ra *Spodoptera exigua* va *Lymantria dispar* hasharot hujayralari liniyalarini laboratoriya sharoitida ko'paytirishning maqbul sharoitlari tanlab olindi. Mazkur sharoitlar tabiiy va sintetik moddalarning insektisid faolligini o'rganishga keng imkoniyat ochib beradi.

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