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COMPARATIVE ANALYSIS OF AFLATOXIN CONTENT IN BITTER ALMOND FRUITS (*PRUNUS DULCIS VAR. ACHCHIQ*) GROWING IN VARIOUS GEOGRAPHICAL REGION UZBEKISTAN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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The aim of the study was to optimize the process of extraction of aflatoxins secreted by fungi of the genus *Aspergillus* from almonds, as well as to analyze the content of aflatoxins using high performance liquid chromatography (HPLC). An HPLC LC 2030 C3D Plus (Shimadzu) column Shim-pack GIST-HP C18 150x4.6 mm 3 μm (Shimadzu) was used at a ratio of $\text{H}_2\text{O}/\text{H}_3\text{C}-\text{CN} = 40/60$ as an eluent at a thermostat temperature of 40 degrees Celsius. Based on the analysis on a fluorescent detector RF-20A with an excitation wavelength of 365-450 nm and a flow rate of 0.5 ml/min, a directed sample volume of 10 μl , the amount of natural compounds AFB1, AGB2, AFG1, AFG2 of the aflatoxin group was determined. It has been established that the content of aflatoxins does not exceed the permissible limits and differs depending on the area of cultivation. Almonds grown in the Syrdarya region contain one and a half times more aflatoxin G2 than almonds grown in the Jizzakh region, and four times more than almonds grown in the Samarkand region.

Keywords: fungi of the genus *Aspergillus*, extraction, qualitative and quantitative analysis

СРАВНИТЕЛЬНЫЙ АНАЛИЗ СОДЕРЖАНИЯ АФЛАТОКСИНОВ В ЯДРАХ ГОРЬКОГО МИНДАЛЯ (*PRUNUS DULCIS VAR. ACHCHIQ*), ПРОИЗРАСТАЮЩИХ В РАЗЛИЧНЫХ ГЕОГРАФИЧЕСКИХ РЕГИОНАХ УЗБЕКИСТАНА, МЕТОДОМ ВЫСОКОЭФФЕКТИВНОЙ ЖИДКОСТНОЙ ХРОМАТОГРАФИИ

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Целью исследования являлась оптимизация процесса экстракции афлатоксинов, выделяемых грибами рода *Aspergillus*, из миндаля, а также анализ содержания афлатоксинов с помощью высокоеффективной жидкостной хроматографии (ВЭЖХ). Использована ВЭЖХ LC 2030 C3D Plus (Shimadzu), колонка Shim-pack GIST-HP C18 150x4,6 мм 3 мкм (Shimadzu) при соотношении $\text{H}_2\text{O}/\text{H}_3\text{C}-\text{CN} = 40/60$ в качестве элюента при температуре терmostата 40 градусов по Цельсию. На основе анализа на флуоресцентном детекторе RF-20A с длиной волны возбуждения – 365-450 нм и расходом 0,5 мл/мин, объемом направленного образца 10 μl определено количество природных соединений AFB1, AGB2, AFG1, AFG2 группы афлатоксинов. Установлено, что содержание афлатоксинов не превышает допустимые нормы и отличается в зависимости от области выращивания. Миндаль, выращенный в Сырдарьинской области, содержит в полтора раза больше афлатоксина G2, чем миндаль, выращенный в Джизакской области, и в четыре раза больше, чем миндаль, выращенный в Самаркандской области.

Keywords: грибы рода *Aspergillus*, экстракция, качественный и количественный анализ

O'ZBEKİSTONNING TURLI GEOGRAFIK HEDUDLARDA O'SADIGAN ACHCHIQ BODOM (*PRUNUS DULCIS VAR. ACHCHIQ*) MEVASI TARKIBIDAGI AFLATOKSINLAR MIQDORINI YUQORI SAMARALI SUYUQLIK XROMATOGRAFIYASI METODI YORDAMIDA QIYOSIY TAHLIL QILISH

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Tadqiqotning maqsadi bodomdan *Aspergillus* jinsi zamburug'lari tomonidan ajratilgan aflatoksinlarni ajratib olish jarayonini optimallashtirish, shuningdek, yuqori samarali suyuqlik xromatografiyasi (HPLC) yordamida aflatoksinlar tarkibini tahlil qilish. HPLC LC 2030 C3D Plus (Shimadzu) ustuni Shim-pack GIST-HP C18 150x4,6 mm 3 mkm (Shimadzu) $\text{H}_2\text{O}/\text{H}_3\text{C}-\text{CN} = 40/60$ nisbatida elyuent sifatida termostat harorati Selsiy bo'yicha 40 darajada ishlatalilgan, 365-450 nm qo'zg'alish to'lgan uzunligi va 0,5 ml/min oqim tezligi, 10 mlk yo'naltirilgan namuna hajmi, AFB1, AGB2, AFG1, AFG2 tabiyti birikmalarining miqdori RF-20A luminesentsen detektorida tahlil qilish asosida aflatoksin guruhi aniqlandi. Aflatoksinlar miqdori ruxsat etilgan chegaralardan oshmasligi va etishitirish maydoniga qarab farq qilishi aniqlangan. Sirdaryo viloyatida yetishtirilgan bodom tarkibida aflatoksin G2 moddasi Jizzax viloyatida yetishtirilgan bodomdan qaranganda bir yarim barobar, Samarqand viloyatida yetishtirilgan bodomdan to'rt barobar ko'p.

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Kirish

Zamburug'lar o'zidan hayotiy xavfli ikkilamchi metabolitlar – mikotoksinlar ajratishi bilan tavsiflanadi. Shunga ko'rta zamburug'lar bilan zararlangan oziq-ovqat mahsulotlari mutagenli, kantserogenli va immunosupressiv xususiyatga ega bo'lgan toksinlarni bol'shi ehtimolligi bilan xavflidir [1, 2]. Oziq-ovqat mahsulotlarida o'zidan mikotoksinlar ajratuvchi mag'or zamburug'ining *Aspergillus*, *Penicillium* va *Fusarium* hisoblanadi. Aksariyat toksin ajratuvchi zamburug'lar *Aspergillus* avlodlari bo'lib, ularning turlari

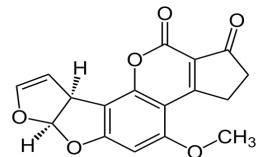
gliotoksin yoki verrukulogen (*A. Fumigatus*), aflatoksinlar (*A. flavus* va *A. parasiticus*) ajratishi bilan xavfli hisoblanadi [3, 4].

Aflatoksinlar *Aspergillus* turlari tomonidan ishlab chiqarilgan ikkilamchi metabolitlar guruhidir. Yutilganda, nafas olganda yoki teri orqali so'rilsa, aflatoksinlar, hatto past konsentratsiyalarda ham, odamlar va hayvonlarda kanserogen, gepatotoksik, teratogen va mutagen xususiyatga ega va shuning uchun Jahon sog'liqni saqlash tashkiloti [5-9] bo'yicha 1-guruh kanserogenlari sifatida tasniflanadi. Aflatoksin B1

ayniqsa muhim, chunki u eng zaharli va kuchli hech qachon tavsiflangan gepatokarsinogen tabiiy birikma [8-13].

Ushbu muammoning muhimligini hisobga olgan holda, aflatoksinlarni aniqlashning turli usullari ishlab chiqilgan, ammo har bir usulning kamchiliklari mavjud: - samarali, ammo qimmat yoki arzon, ammo mehnatkash va etarlicha aniq emas [9-12].

Aspergillus turkumiga oid zamburug'lar ajratadigan Aflatoksin guruhi nomi bilan tavsiflanadigan mikotoksinlar nafas yo'lli yoki organizmning ichki a'zolariga tushishi bilan shilimshiq epitelial to'qima orqali so'rili ikkilamchi metabolitlar sifatida kantserogenli, gepatotoksik, teratogen va mutagen ta'sir etish xususiyati bilan kantserogen guruhiga kiritiladi [5-7, 14-16]. Ushbu ikkilamchi metabolitlar sifatida qayd etilgan mikotoksinlardan Aflatoksin B1 zaharli va gepatokartsinogen sifatida kuchli ta'sir etuvchi tabiiy birikma hisoblanadi [8].



B1 aflatoksin 5 ta halqadan iborat strukturaviy tuzilishga ega, kimyoiy formulasi $C_{17}H_{12}O_6$, molekulyar massasi 312,2798 g/mol, suyuqlanish harorati 269 °C, svuda eruvchanligi 10-20 mkg/ml bo'lib, agregat holati rangsiz yoki och sariq kristalldir Kimyoiy formulasi $C_{17}H_{12}O_6$, molekulyar massasi 312,2798 g/mol, suyuqlanish harorati 269 °C, svuda eruvchanligi 10-20 mkg/ml bo'lib agregat holati rangsiz yoki och sariq kristalldir [8].

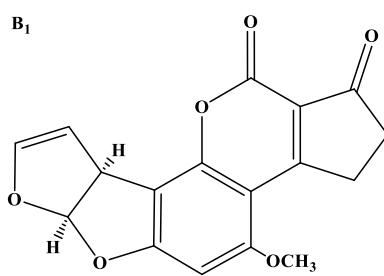
Aflatoksinlar guruhiga kiruvchi birikmalarning oziq-ovqat va ularning manbalarida mavjudligi hamda miqdorining aniqlash muhimligi bois aniqlashning har xil metodlari ishlab chi-

qilgan va shuningdek ushbu metodlar asosida natijalarda aniqliklar etishmasligi sababli kamchiliklarga ham ega [9-16].

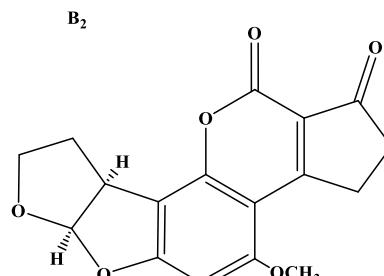
Oziq-ovqat va ozuqalarning erkin savdosи butun dunyoda tobora keng tarqalgan. Mahsulot oqimini nazorat ostida ushlab turish uchun eksport qiluvchi va import qiluvchi mamlakatlarda ham muvofiqlashtirilgan tartibga solish va boshqarish tizimlariga ehtiyoj bor. Shu sababli, ko'plab mamlakatlarda allaqachon turli xil ifloslantiruvchi moddalar, shu jumladan aflatoksinlar uchun umumiyligida qoidalalar va maksimal darajalar o'rnatilgan. Shunga qaramay, ayrim davlatlar aflatoksinlar uchun o'zlarining maksimal darajalariga ega. Dunyo bo'y lab asosan aflatoksinlarning umumiyligida miqdori (AFB1, AFB2, AFM1, AFG1 va AFG2), oziq-ovqat va ozuqa uchun, muvofiqlikni tekshirish uchun tegishli tahlil usullarini tanlash uchun boshqalar qatori ushbu qoidalardan xabaridor bo'lislidir. Hozirgi kunda aflatoksinlarning aniqlashda ommalashgan, zamona viy usullardan foydalanib, aflatoksinlarni miqdor va sifat jihatdan aniqlash usulidan yuqori samarali suyuqlik xromatografiyasidan foydalandi [12].

Yuqori samarali suyuq xromatografiya (YSSX) - bu organik birikmalarni ajratish va aniqlashning eng ommaviy va samarali usullaridan hisoblanadi. Dunyoda taxminan 80% organik birikmalar YSSX yordamida aniqlanadi. Tahlil qilinadigan namuna kolonkada harakatli va harakatsiz fazalar o'rtasida harakatlanadi va taqsimlanadi. Bunda ajratish kerak bo'lgan namunalar tarkibidagi komponentlar ikki faza uchun turli xil yaqinliklarga ega bo'lganligi va shu bilan kolonkada har xil tezlikda harakatlanishi sababli ajratiladi [13].

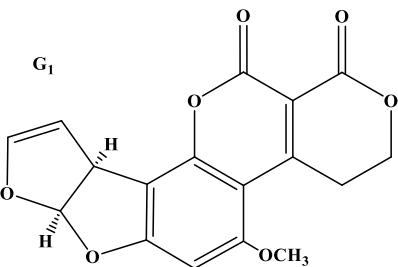
B₁



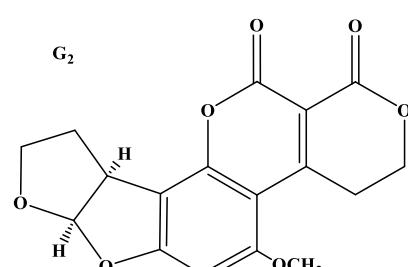
B₂



G₁



G₂



Aflatoksin B1, B2, G1 va G2 larning tuzilishi

Ob'ektlar va tadqiqot usullari

Tadqiqot ob'ekti sifatida O'zbekiston Respublikasi Sirdaryo Xovos Tumani Sohibkor MFY, Jizzax viloyat G'allaorol tumani va Samarqand viloyati Payariq tumanlarida o'sadigan achchiq bodomning (*Prunus dulcis* var. *Achchiq*) 2020 yilgi yetishtirilgan hosilidan namunalar olindi. Hosil namunalari 0,1 smdan kichik o'lchamgacha blendir yordamida maydalandi. Olingan namunalar blenderda maydalangandan so'ng ekstraksiya qilindi [14, 15].

Optimal ekstaksiya quyidagicha amalga oshirildi. Bunda suv va asetonitrildan teng nisbatda olinib 125 ml eritma tayyorlab, unga 5 g natriy xlorid tuzidan solinib yaxshilab aralashtirildi. Tuz erigandan so'ng, namunada 25 g tortilib tayyor eritmaga solinib magnit aralashtirgichda 15 min davomida aralashtirib ekstraksiya qilinadi[16]. Bodom namunalari ekstraksiya qilingandan so'ng 0,45 μ mli filtrda filtrlab olinib, namunalar tarkibidagi aflatoksinlar miqdorini aniqlashda yuqori samarali suyuqlik xromatografiya (YSSX) usulidan foydalanildi. Buning uchun namunalar ekstrakti filtr qilgandan so'ng YSSX qurilmasiga analiz qilish uchun joylashtiriladi [17].

Analizni bajarishimiz uchun bizda

"Konstanta" xususiy ishlab chiqaruvchi tomonidan 1 – partiyasida ishlab chiqarilgan Aflatoksin G1 uchun O'z DSN 03.3137;2020, Aflatoksin G2 uchun O'z DSN 03.3141;2020, Aflatoksin B2 uchun O'z DSN 03.3142;2020 va Aflatoksin B1 uchun O'z DSN 03.3140;2020 Pasport seriyali standartlardan foydalanib YSSX LC 2030 C3D Plus (Shimadzu) qurilmasi Shim-pack GIST-HP C18 150x4,6 mm 3 μ m (Shimadzu) kolonkadan foydalanilib, termostat harorati 40 °C da elyuent sifatida H₂O/H₃C-CN - 40/60 nisbatda olindi. Fluorescent RF-20A detektorda qo'zg'aluvchan to'lqin uzunligi - 365 - 450 nmda va 0,5 ml/min oqim tezligida, yuborilgan namuna hajmi 10 mkl da analiz amalga oshirildi.

Natijalar va muhokama

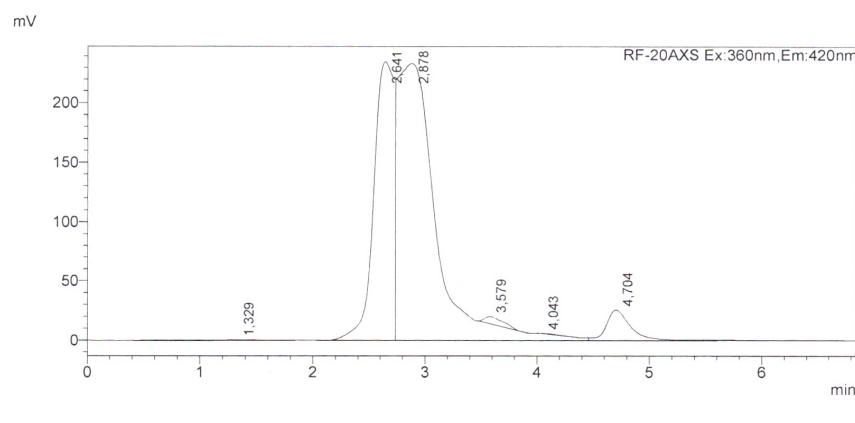
Turli geografik mintaqalardan keltirilgan namunalar yuqori samarali suyuqlik xromatografiyasini qurilmasida tahililar amalga oshirildi. Unga ko'ra Samarqand viloyatida yetishtirilgan bodom namunasi tarkibida aflatoksin miqdori kamroq ekanligi yuqori samarali suyuqlik xromatografiysida bajarilgan analiz natijasida aniqlandi. Buni quyida keltirilgan (1-jadval va 1-rasm).

Sirdaryo va Jizzax viloyatlarida yetishtirilgan bodomlarda aflatoksinlar miqdorini tahlil qilish uchun joylashtiriladi [17].

1-jadval

Samarqand viloyatida yetishtirilgan bodom tarkibidagi aflatoksin miqdori

Peak	Ret.Time	Area	Height	Conc	Unit	Mark	Name
1	1,329	16505	376	0,000	ug/ml		AF1 G2
2	2,641	2976073	235015	0,000		V	
3	2,878	5535576	233477	0,000		SV	
4	3,579	77056	6102	0,001		T	
5	4,043	7633	432	0,000		T	
6	4,704	404106	25654	0,000		SV	
Total		9016949	501056				

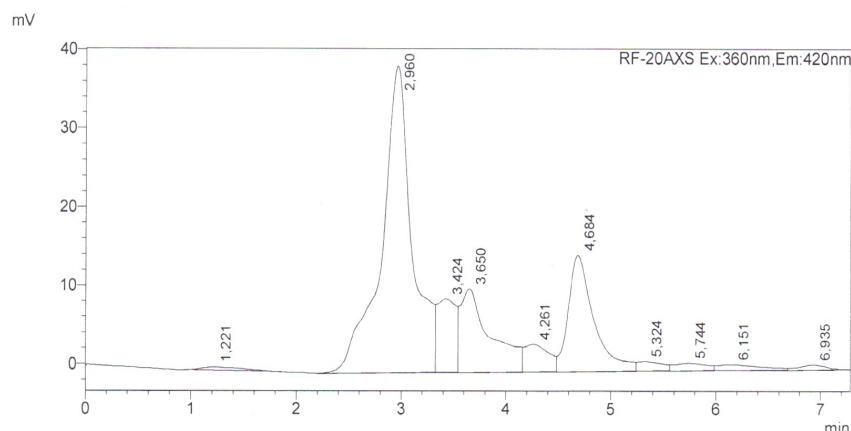


1-rasm. Samarqand hududida yetishtirilgan bodom namuna tarkibi xromatogrammasi.

2-jadval

Sirdaryo viloyatida yetishtirilgan bodom tarkibidagi aflatoksin miqdori

Peak	Ret.Time	Area	Height	Conc	Unit	Mark	Name
1	1,221	12224	439	0,000	ug/ml		AF1 G2
2	2,960	789877	39014	0,000			
3	3,424	116271	9404	0,000		V	
4	3,650	211071	10649	0,004		V	
5	4,261	56245	3483	0,000		V	
6	4,684	250917	14809	0,000		V	
7	5,324	19973	1200	0,000		V	
8	5,744	21369	976	0,000		V	
9	6,151	22771	753	0,000		V	
10	6,935	12249	663	0,000		V	
Total		1512967	81390				



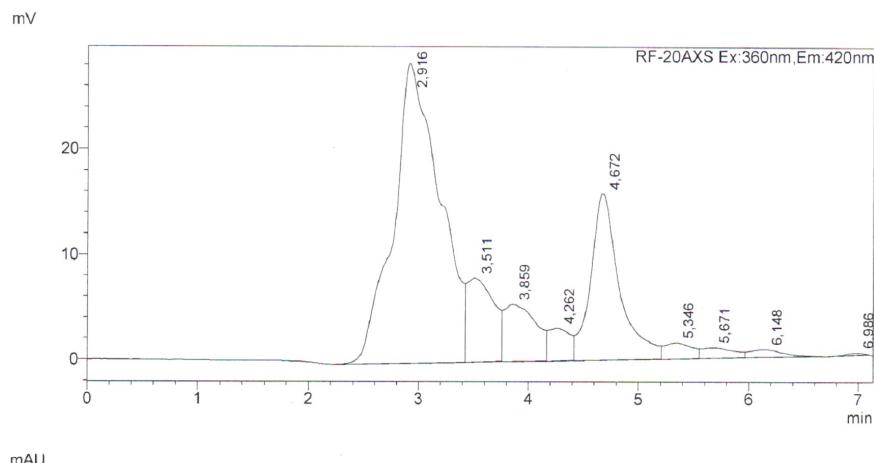
2-rasm. Sirdaryo viloyatida yetishtirilgan bodom namuna tarkibi xromatogrammasi.

lish natijalari 2 va 3-jadvallarda hamda 2 va 3-rasmlarda keltirilgan.

3-jadval

Jizzax viloyatida yetishtirilgan bodom tarkibidagi aflatoksin miqdori

Peak	Ret.Time	Area	Height	Conc	Unit	Mark	Name
1	2,916	836053	28468	0,000	ug/ml		AF1 G2
2	3,511	135789	8015	0,003		V	
3	3,859	108846	5431	0,000		V	
4	4,262	42028	3053	0,000		V	
5	4,672	287502	15852	0,000		V	
6	5,346	25890	1500	0,000		V	
7	5,671	19997	979	0,000		V	
8	6,148	15801	734	0,000		V	
9	6,986	2713	236	0,000			
Total		1474619	64269				



3-rasm. Jizzax viloyati hududida yetishtirilgan bodom mevasi xromatogrammasi.

Turli geografik mintaqalardan keltirilgan tadqiqot ob'ekti tarkibining qiyosiy tahlili asosida qayd etish mumkinki, garchi mahsulotlar bir avlodga mansub o'simlik turi bo'lsada, ularni meva-

sida uchrovchi zamburug'lar miqdori va ulardan ajraladigan toksinlarga ko'r'a alohida baho berish mumkin. Olingan natijalar quyidagi 4-jadvalda keltirilgan.

4-jadval

O'zbekistonning turli geografik mintaqalarda o'sadigan achchiq bodom mevasida zamburug'ladan ajraladigan aflatoksinlar ko'rsatkichlari

Namuna keltirilgan hududlar	Aflatoksinlarning umumiyligi miqdori chegarasi aflatoksinlarning umumiyligi tarkibiga qo'yiladigan talablar [18] ppm	AFB1	AFB2	AFG1	AFG2	Aflatoksinlarning umumiyligi miqdori ppm
Samarqand Payariq tumani	0,01	-	-	-	0,001	0,001
Jizzax G'allaorol tumani		-	-	-	0,003	0,003
Sirdaryo Xovos tumani		-	-	-	0,004	0,004
Afg'aniston		-	-	-	0,001	0,001
Eron		-	-	-	0,003	0,003

Olingan natjalarga asosan, shuni qayda etish mumkinki, Ozbekiston Respublikasi turli viloyatlarida yetishtirilgan bodom mevasi tarkibida aflatoksinlar miqdori keltirilgan me'yoriy hujjatlarga nisbatan kam. Aflatoksin B1, B2, G1 va G2 standart namunalarini asosida 3,65 – minutda aniqlandi. Sirdaryo hududida yetishtirilgan bodom mevasi tarkibidagi aflatoksin G2 miqdori, Jizzax viloyatida yetishtirilgan bodom mevasi tarkibidagi aflatoksin G2 ga nisbatan bir yarim barobar, Samarqand viloyatida yetishtirilgan bodom mevasiga nisbatan esa to'rt barobar yuqori ekan.

Xulosa

YSSX LC 2030 C3D Plus (Shimadzu) qurilmasi Shim-pack GIST-HP C18

150x4,6 mm 3 μm (Shimadzu) kolonidan foydalаниlib, termostat harorati 40 °C da elyuent sifatida $\text{H}_2\text{O}/\text{H}_3\text{C}-\text{CN} = 40/60$ nisbatda olindi. Fluorescent RF-20A detektorda qo'zg'aluvchan to'lqin uzunligi - 365 - 450 nm da 0,5 ml/min oqim tezligida, yuborilgan namuna hajmi 10 mklda analiz amalga oshirilish asosida aflatoksin guruhidagi AFB1, AGB2, AFG1, AFG2 tabiiy birikmalarni aniqlashning optimal usuli aniqlandi.

Aflatoksinlarni bodom o'simligi mag'zidan ajratib olishni optimallashtirish, shuningdek aflatoksinlarni oson va samarali ekstraksiya qilib ajratish, hamda O'zbekiston Respublikasi uchta viloyatida yetishtirilgan achchiq bodom daraxti mag'zi tarkibidagi aflatoksinlari miqdori tahlil qilindi.

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