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Rare and endemic plant species of the flora of Kostanay Region

Based on research conducted in the territory of Kostanay Region from 1995 to 2025, eight endemic plant species belonging to five families and seven genera have been recorded in the region. Rare and endangered plant species listed in the Red Book of the Republic of Kazakhstan are represented in Kostanay Region by 18 taxa: *Convallaria majalis* L., *Ornithogalum fischerianum* Krasch., *Alnus glutinosa* (L.) Gaertn., *Betula tianschanica* Rupr., *Dianthus capitatus* subsp. *andrzejowskianus* Zapał., *Drosera rotundifolia* L., *Chimaphila umbellata* (L.) W.P.C. Barton, *Lilium martagon* L., *Tulipa sylvestris* subsp. *australis* (Link) Pamp., *Tulipa suaveolens* Roth., *Nymphoides peltata* (S.G. Gmel.) Kuntze, *Dactylorhiza maculata* subsp. *fuchsii* (Druce) Hyl., *Epipactis palustris* (L.) Crantz., *Koeleria macrantha* subsp. *macrantha*, *Stipa pennata* L., *Adonis volgensis* Stev., *Pulsatilla patens* (L.) Mill., *Pulsatilla patens* subsp. *flavescens* (Zucc.) Zämelis (= *Pulsatilla flavescens* (Zucc.) Juz.). The data were obtained through a review of literature sources and during numerous botanical expeditions conducted by the authors in the Kostanay Region. All collected specimens are deposited in the Herbarium of Kostanay Regional University (KSPI). This article presents precise locality data for endemic and rare plant species of the region, which may contribute to improving their protection and to the development of targeted conservation measures.

Keywords: location data, flora revision, rare plant species, endemic plant species, flora of the Republic of Kazakhstan, flora of Kostanay Region, Red Book of Kazakhstan, international herbaria.

Introduction

Issues of plant endemism and the protection of rare and endangered species are key elements in floristic research for any region, including the Republic of Kazakhstan (RK). In recent decades, opportunities for revising flora have significantly increased. Firstly, due to the online publication of collections from some of the world's richest herbaria, such as the Komarov Botanical Institute (LE) and Lomonosov Moscow State University (MW). Secondly, through the emergence of websites like IPNI (since 1999) and GBIF (since 2001), which provide access to global biodiversity data on flora and fauna.

As a result of a more detailed study of species distributions, we clarified the ranges of many plants. It was often found that some species are much more widely distributed than previously known during the compilation of The Flora of Kazakhstan (1956–1964) [1] and the last major floristic review of Kostanay Region [2]. Accordingly, certain species previously considered endemic to the Republic of Kazakhstan are no longer recognized as such. At the same time, new species have been discovered, whose known distribution is limited to the territory of Kazakhstan [3, 4]. Therefore, the list of endemic species of Kazakhstan within the region, published by the authors, differs significantly from earlier records.

The list of rare species occurring in Kostanay Region and included in the Red Book of Kazakhstan was also considerably revised due to the most recent edition of the Red Book and changes in nomenclature. The results of the floristic revision of the region are also reflected in several previously published articles [5–12].

Experimental

Sampling Methods

The material was obtained through the study of the following herbaria: A. Baitursynuly Kostanay Regional University (acronym: KSPI), Komarov Botanical Institute (LE), Lomonosov Moscow State University (MW) [13], Institute of Plant and Animal Ecology, Ural Branch of RAS (SVER), Institute of Botany and Phytointroduction (AA), as well as official websites: International Plant Names Index (IPNI) [14], Global Biodiversity Information Facility (GBIF) [15], Plants of the World Online (POWO) [16]. The taxonomic position and distribution of published species were clarified using the above-mentioned sites. Herbarium da-

tabases were utilized to compile a comprehensive species list based on updated taxonomic data. As a result of the analysis of current distribution data, the authors determined the status of each species.

A database was created summarizing families and species. For each species, the Latin name is provided according to the International Plant Names Index (IPNI) [14]. The label text from herbarium specimens was used to determine collection dates and precise locations.

The final, verified checklist of vascular plants of Kostanay Region (1,145 species from 452 genera and 101 families) was published by the authors in 2024 [17].

Geographic Coverage

General Information: Kostanay Region is an administrative area in northern Kazakhstan, covering 196,001 km², with a population of 864,550 (as of 2022).

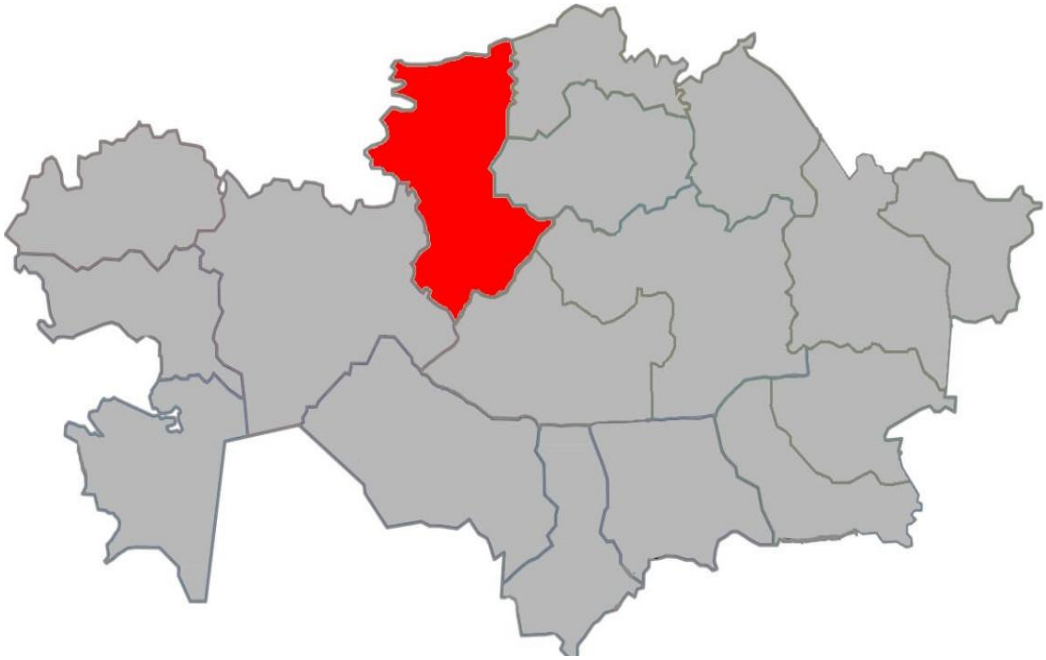

Figure 1. Kostanay Region on the map of Kazakhstan

In Figure 1, Kostanay Region is marked in red in the north of the Republic of Kazakhstan. Geographical coordinates of the region: center (Kostanay city): 53°12'00" N, 63°38'00" E, north (Zverinogolovoe village): 54°45' N, 64°86' E, south (Torgai village): 49°63' N, 63°49' E, west (Zhitikara city): 52°16' N, 61°22' E, east (Sarykol village): 53°31' N, 65°53' E [18].

Relief: most of the north of the region is occupied by the southwestern edge of the West Siberian Lowland, to the south of it is the Turgai plateau; in the west of the region is the undulating plain of the Trans-Ural plateau, and in the southeast are the spurs of the Sary-Arka. The territory is characterized by a relatively flat terrain. The average altitude above sea level varies from 200 to 400 m [17].

Results and Discussion

Taxonomic Coverage

The vascular plant flora of Kostanay Region includes 1,145 species, 452 genera, and 101 families. The taxonomic structure is dominated by the division Magnoliophyta, with a small proportion of pteridophytes and gymnosperms. The ratio of monocots to dicots in Magnoliophyta is approximately 1:3.8. The leading family in the flora is Asteraceae Dumort. (196 species), followed by Poaceae Barnhart — 91 species, Fabaceae Lindl. — 76 species, Amaranthaceae Juss. — 75 species, Brassicaceae Burgett — 62 species, Caryophyllaceae Juss. — 49 species, Cyperaceae Juss. — 43 species, Rosaceae Juss. — 42 species, Plantaginaceae Juss. — 32 species, Apiaceae Lindl. — 32 species. The composition of leading families reflects the general floristic patterns typical of the arid and semi-arid zones of Kazakhstan [17].

Traits Coverage

In the Kostanay Region, 8 species endemic to Kazakhstan, belonging to 5 families and 7 genera, were identified based on the revision of endemic vascular plants of Kazakhstan [12]. The Red Book of the Republic of Kazakhstan includes rare and endangered species, 18 of which are found in this region (Tab. 1).

Table 1

Endemic, Rare, and Endangered Species in the Territory of Kostanay Region

№	Family	Species name	Herbarium	Conservation Status	Endemism
1	Asteraceae	*Artemisia camelorum* Krasch.	MW	Not Evaluated	ED RK
2	Asteraceae	*Jurinea transuralensis* Iljin	AA	Not Evaluated	ED RK
3	Amaranthaceae	*Climacoptera turgaica* (Iljin) Botsch.	MW	Not Evaluated	ED RK
4	Amaranthaceae	*Petrosimonia hirsutissima* (Bunge) Iljin	MW	Not Evaluated	ED RK
5	Asparagaceae	*Convallaria majalis* L.	KSPI	Vulnerable, KK RK	not ED
6	Asparagaceae	*Ornithogalum fischerianum* Krasch.	KSPI	Vulnerable, KK RK	not ED
7	Betulaceae	*Alnus glutinosa* (L.) Gaertn.	KSPI	Vulnerable, KK RK	not ED
8	Betulaceae	*Betula saviczii* V.N. Vassil.	LE	Not Evaluated	ED RK
9	Betulaceae	*Betula tianschanica* Rupr.	KSPI	Vulnerable, KK RK	not ED
10	Caryophyllaceae	*Dianthus capitatus subsp. andrzejowskianus* Zapał	KSPI	Vulnerable, KK RK	not ED
11	Droseraceae	*Drosera rotundifolia* L.	MW	Critically Endangered	not ED
12	Ericaceae	*Chimaphila umbellata* (L.) W.P.C. Barton	KSPI	Vulnerable, KK RK	not ED
13	Fabaceae	*Astragalus chaetolobus* Bunge	MW	Not Evaluated	ED RK
14	Liliaceae	*Tulipa auliekolica* Perezhogin	LE	Not Evaluated	ED RK
15	Liliaceae	*Tulipa turgaica* Perezhogin	LE	Not Evaluated	ED RK
16	Liliaceae	*Tulipa sylvestris subsp. australis* (Link) Pamp.	SVER	Vulnerable, KK RK	not ED
17	Liliaceae	*Tulipa suaveolens* Roth	KSPI	Vulnerable, KK RK	not ED
18	Liliaceae	*Lilium martagon* L.	KSPI	Critically Endangered	not ED
19	Menyanthaceae	*Nymphoides peltata* (S.G. Gmel.) Kuntze	KSPI	Vulnerable, KK RK	not ED
20	Orchidaceae	*Dactylorhiza maculata subsp. fuchsii* (Druce) Hyl.	KSPI	Vulnerable, KK RK	not ED
21	Orchidaceae	*Epipactis palustris* (L.) Crantz	KSPI	Vulnerable, KK RK	not ED
22	Poaceae	*Koeleria macrantha* (Ledeb.) Schult	MW	Vulnerable, KK RK	not ED
23	Poaceae	*Stipa pennata* L.	SVER	Vulnerable, KK RK	not ED
24	Ranunculaceae	*Adonis volgensis* Stev.	SVER	Vulnerable, KK RK	not ED
25	Ranunculaceae	*Pulsatilla patens* (L.) Mill.	KSPI	Vulnerable, KK RK	not ED
26	Ranunculaceae	*Pulsatilla patens subsp. flavescens* (Zucc.) Zämelis	KSPI	Vulnerable, KK RK	not ED
KK RK — Red Book of Republic of Kazakhstan, ED RK — Endemic of Republic of Kazakhstan					

The following species (Tab. 2) from the Orchidaceae family grow in the territory of Kostanay Region and are recommended for inclusion in the new edition of the “Red Book of the Republic of Kazakhstan”.

Table 2

List of Orchidaceae species occurring in the territory of Kostanay Region

№	Species name	Herbarium	Instance collection point	Conservation Status	Endemism
1	*Dactylorhiza incarnata* (L.) Soó	MW	Naurzum State Nature Reserve, 20 km east of Aksuat village	absent	not ED
2	*Dactylorhiza incarnata subsp. cilicica* (Klinge) H.Sund.	MW	Naurzum State Nature Reserve, 5 km southwest of Aksuat village	absent	not ED
3	*Dactylorhiza salina* (Turcz. ex Lindl.) Soó	MW	Naurzum State Nature Reserve, wet meadow near spring outlets, north of the Naurzum pine forest	absent	not ED
4	*Epipactis atrorubens* (Hoffm.) Bess.	LE, KSPI	Uzunkol District, vicinity of KrasnyeBorki village	absent	not ED
5	*Gymnadenia conopsea* (L.) R. Br.	LE	Semiozyorny District, Aman-Karagay forest, edge of a salt flat	absent	not ED
6	*Malaxis monophyllos* (L.) Sw.	LE	Mendykara District, mossy area near a spring by the lake	absent	not ED
7	*Spiranthes australis* (R.Br.) Lindl.	MW	Mendykara District, mossy lake near Borovoye village	absent	not ED

Temporal Coverage

The study of vascular plant flora in Kostanay Region spans approximately a 100-year period. The collections conducted by A.G. Voronov, F.N. Rusanov, and others in the Naurzum Nature Reserve during the first half of the 20th century are of particular historical and scientific importance. Herbarium specimens from that period are preserved in Russia, in Saint Petersburg, at the Herbarium of the Komarov Botanical Institute (LE), as well as at the Herbarium of Lomonosov Moscow State University (MW). Later collections from the Naurzum Reserve are also stored at MSU's Herbarium (MW). Collections from the late 20th century, led by Professor P.G. Pugachev, were primarily carried out by students of Kostanay Pedagogical University and are labeled accordingly. Over the past 30 years, the authors of this article, researchers from Kostanay Regional University (KRU), namely Y.V. Perezhogin, Zh.T. Suyundikova, O.V. Borodulina, and others, have studied the flora of Kostanay Region. Their materials are stored in the herbarium of A. Baitursynuly Kostanay Regional University (acronym: KSPI).

Usage Licence

Data Resources

Number of data packages: 2

Titles of data packages: 1. Endemic and Rare Species of Kostanay Region, 2. List of Orchidaceae Species Occurring in Kostanay Region

Data Format: CSV

Description: The databases contain species lists, including 26 endemic and rare species (1st database) and 7 species from the Orchidaceae family (2nd database). Each entry includes information on taxonomic names, the names of herbaria where specimens are stored, collector names and specimen numbers, precise collection locations in nature, conservation status, and endemism within the Republic of Kazakhstan (Tab. 3).

Table 3

Column Descriptions for Plant Species Dataset

Column Label	Column Description
Family	Plant family
Genus	Plant genus
Species	Species epithet
Author	Author of the species name
Herbarium	Abbreviation of the herbarium where the specimen is stored
Collector Number	Serial number assigned to a specific collection by the botanist or collector group
Collector Name	Name(s) of the person(s) who collected the herbarium specimen
Collection Time	Collection date, as indicated on the herbarium label
Instance Collection Point	Description of the specific location where the specimen was collected
Conservation Status	Conservation status according to the Red Book of Plants of the Republic of Kazakhstan
Endemism	Indicates whether the species is endemic to the Republic of Kazakhstan or not

Conclusions and Recommendations

1. Eight endemic species from five families and seven genera grow in the territory of Kostanay Region.

2. Two species previously listed as endemic in The Flora of Kazakhstan have been synonymized and are no longer considered endemic: *Betula kirghisorum* Sawicz and *Linaria dolichocarpa* Klokov.

3. *Astragalus kustanaicus* Popov, Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 10: 16 (1947). Naurzum State Nature Reserve, steppe, 05.06.2009, collected by students of Kostanay State Pedagogical Institute (KSPI01892). Kostanay Region, Semiozyorny (= Auliekol) District, 1.8 km southwest of the Dievsky state farm, 09.06.1964, Samorodov Yu., Lodinova T. (MW0846220).

Listed in POWO as an endemic species of the Republic of Kazakhstan. However, it is not endemic, as it was also found in Orenburg Region, Russian Federation (Orenburg Oblast, Sol-Iletsk District, Troitsky Chalk Hills, slope of a chalk hill, 05.06.2015) [19].

4. Rare and endangered plant species listed in the Red Book of the Republic of Kazakhstan are represented in Kostanay Region by 18 taxa (species and subspecies): *Convallaria majalis* L., *Ornithogalum fischerianum* Krasch., *Alnus glutinosa* (L.) Gaertn., *Betula tianschanica* Rupr., *Dianthus capitatus* subsp. *andrzejowskianus* Zapał., *Drosera rotundifolia* L., *Chimaphila umbellata* (L.) W.P.C. Barton, *Lilium martagon* L., *Tulipa sylvestris subsp. australis* (Link) Pamp., *Tulipa suaveolens* Roth., *Nymphoides peltata* (S.G. Gmel.) Kuntze, *Dactylorhiza maculata subsp. fuchsii* (Druce) Hyl., *Epipactis palustris* (L.) Crantz, *Koeleria macrantha subsp. macrantha*, *Stipa pennata* L., *Adonis volgensis* Stev., *Pulsatilla patens* (L.) Mill., *Pulsatilla patens subsp. flavescens* (Zucc.) Zämelis (= *Pulsatilla flavescens* (Zucc.) Juz.).

5. The following species from the Orchidaceae family grow in Kostanay Region and are recommended for inclusion in the next edition of the "Red Book of the Republic of Kazakhstan": *Dactylorhiza incarnata* (L.) Soó, *Dactylorhiza incarnata subsp. cilicica* (Klinge) H. Sund., *Dactylorhiza salina* (Turcz. ex Lindl.) Soó, *Epipactis atrorubens* (Hoffm. ex Bernh.) Bess., *Gymnadenia conopsea* (L.) R.Br., *Malaxis monophyllos* (L.) Sw., and *Spiranthes australis* (R.Br.) Lindl.

6. The results of this research provide a revised checklist that will contribute to updating information on rare and endemic plants in the forthcoming edition of The Flora of Kazakhstan.

Conflict of interest

The authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript: **Perezhogin Yu.V. —** conceptualization, investigation, methodology, data collection; **Borodulina O.V. —** data curation, analysis, supervision, writing draft; **Suyundikova Zh.T. —** conducting the fieldwork and sample collection.

References

1 Флора Казахстана / ред. Н. В. Павлов. — 1956–1964. — Т. 1–9. — Алма-Ата: Изд-во АН КазССР.

2 Заугольнова Л. Б. Флора и растительность Наурзумского заповедника / Л. Б. Заугольникова, П. Г. Пугачев // Сб. «Флора и растительность Наурзумского государственного заповедника». — Кустанай, 1975. — С. 25–141.

3 Пережогин Ю. В. Новые виды тюльпанов из Северного Казахстана / Ю. В. Пережогин // Ботанический журнал. — 2013. — Т. 98, № 12. — С. 1558–1563.

4 Пережогин Ю. В. Валидизация двух недействительно обнародованных названий видов *Tulipa* L. (*Liliaceae*) из Северного Казахстана / Ю. В. Пережогин // Новости систематики высших растений. — 2014. — Т. 45. — С. 145.

5 Пережогин Ю. В. Дополнение к флоре Казахстана / Ю. В. Пережогин, П. В. Куликов, С. И. Курлов // Ботанический журнал. — 2015. — Т. 100, № 5. — С. 501–503.

6 Пережогин Ю. В. Дополнения и изменения к списку редких и исчезающих растений Костанайской области (Северный Казахстан) / Ю. В. Пережогин, С. И. Курлов // Вестник Оренбургского государственного университета. — 2015. — № 10 (185). — С. 43–47.

7 Пережогин Ю. В. Дополнение к флоре государственного природного резервата «Алтын Дала» / Ю. В. Пережогин, С. И. Курлов // Ботанический журнал. — 2016. — Т. 101, № 3. — С. 302–308.

8 Пережогин Ю. В. Новые виды флоры Казахстана / Ю. В. Пережогин, П. В. Куликов // Вестник Оренбургского государственного университета. — 2017. — № 3 (203). — С. 78–80.

9 Пережогин Ю. В. Флористические находки на территории Костанайской области / Ю. В. Пережогин // Вестник Костанайского государственного педагогического института. — 2020. — № 2 (58). — С. 73–78.

10 Пережогин Ю. В. Флористические находки на территории Костанайской области (часть 2) / Ю. В. Пережогин, Н. Г. Ерохин // Вестник Костанайского государственного педагогического института. — 2020. — № 3 (59). — С. 35–40.

11 Пережогин Ю. В. Флористические находки на территории государственного природного резервата «Алтын Дала» / Ю. В. Пережогин, Н. Г. Ерохин, К. Т. Баймаганбетова // Вестник Костанайского государственного педагогического института. — 2020. — № 3 (59). — С. 40–44.

12 Kubentayev S. A. Revised checklist of endemic vascular plants of Kazakhstan / S. A. Kubentayev, D. T. Alibekov, Yu. V. Perezhogin, G. A. Lazkov, A. N. Kupriyanov, A. I. Ebel, K. S. Izbastina, O. V. Borodulina, B. B. Kubentayeva // PhytoKeys. — 2024. — No. 238. — P. 241–279. https://doi.org/10.3897/phytokeys.238.114475

13 Серегин А. П. (ред.) Коллекция «Гербарий МГУ» [Электронный ресурс] / А. П. Серегин (ред.) // Депозитарий живых систем «Ноев Ковчег» (направление «Растения»). — М.: МГУ, 2020. — Режим доступа: https://plant.depo.msu.ru/module/collectionpublic? d=P&openparams=%5Bopen-id%3D1524305 %5D

14 International Plant Names Index (IPNI). [Electronic resource]. — 1999. — Access mode: https://www.ipni.org

15 Global Biodiversity Information Facility [Electronic resource]. — 2001. — Access mode: https://www.gbif.org

16 Plants of the World Online [Electronic resource]. — 2017. — Access mode: https://www.ipni.org

17 Пережогин Ю. В. Сосудистые растения Костанайской области (конспект, анализ и ДНК-баркодирование флоры): монография / Ю. В. Пережогин, О. В. Бородулина, С. И. Курлов, А. А. Какимжанова, Ш. А. Манабаева, А. Б. Шевцо. — Астана: Центр Элит, 2024. — 464 с.

18 Костанайская область // Википедия: свободная энциклопедия. — [Электронный ресурс]. — 2004. — Режим доступа: https://ru.wikipedia.org/wiki/Костанайская_область (Дата обращения: 6 июня 2025 г.).

19 Голованов Я. М. Изображение *Astragalus kustanaicus* Popov / Я. М. Голованов // Плантариум. Растения и лишайники России и сопредельных стран: открытый онлайн-атлас и определитель растений. — Электронный ресурс. — Режим доступа https://www.plantarium.ru/page/image/id/479834.html (Дата обращения: 6 июня 2025 г.).

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Қостанай облысының флорасындағы сирек және эндемикалық өсімдік түрлері

1995–2025 жылдар аралығында Қостанай облысы аумағында (Солтүстік Қазақстан) жүргізілген көпжылдық зерттеулер нәтижесінде, бұл өңірде Қазақстан Республикасына ғана тән 5 тұқымдас пен 7 туысқа жататын 8 эндемикалық өсімдік түрі өсетіні анықталды. Еліміздің Қызыл кітабына енгізілген сирек және жойылып бара жатқан өсімдіктер түрлері Қостанай облысында 18 таксонмен (түрлер және түртармақтары) көрсетілген, атап айтқанда: *Convallaria majalis* L., *Ornithogalum fischerianum* Krasch., *Alnus glutinosa* (L.) Gaertn., *Betula tianschanica* Rupr., *Dianthus capitatus* subsp. *andrzejowskianus* Zapał., *Drosera rotundifolia* L., *Chimaphila umbellata* (L.) W.P.C. Barton, *Lilium martagon* L., *Tulipa sylvestris* subsp. *australis* (Link) Pamp., *Tulipa suaveolens* Roth., *Nymphoides peltata* (S.G. Gmel.) Kuntze, *Dactylorhiza maculata* subsp. *fuchsii* (Druce) Hyl., *Epipactis palustris* (L.) Crantz., *Koeleria macrantha* subsp. *macrantha*, *Stipa pennata* L., *Adonis volgensis* Stev., *Pulsatilla patens* (L.) Mill., *Pulsatilla patens* subsp. *flavescens* (Zucc.) Zämelis (= *Pulsatilla flavescens* (Zucc.) Juz.). Материалдар Қостанай облысы

аумағындағы әдеби дереккөздерді талдау және авторлардың көптеген ботаникалық экспедициялары нәтижесінде жиналды. Барлық материалдар Ахмет Байтұрсынұлы атындағы Қостанай өңірлік университетінің Гербарий қорында (қысқартылған атауы — KSPI) сақталған. Мақала Қостанай облысы аумағында өсетін сирек және эндемиялық өсімдіктердің таралуы туралы нақты мәліметтерді ұсына отырып, олардың тиімді және нысаналы қорғау шараларын әзірлеуге мүмкіндік береді.

Кілт сөздер: орналасқан жері туралы мәліметтер, флораларды санау, өсімдіктердің сирек түрлері, өсімдіктердің эндемиялық түрлері, Қазақстан Республикасының флорасы, Қостанай облысының флорасы, Қазақстан Республикасының Қызыл кітабы, халықаралық Гербарийлер

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Редкие и эндемичные виды растений флоры Костанайской области

В результате многолетних исследований на территории Костанайской области (Северный Казахстан) в 1995–2025 гг. выяснилось, что на ней произрастают 8 эндемичных (для Республики Казахстан) видов из 5 семейств и 7 родов. Редкие и исчезающие виды растений, внесенные в Красную книгу Республики Казахстан, на территории Костанайской области представлены 18 таксонами (виды и подвиды): *Convallaria majalis* L., *Ornithogalum fischerianum* Krasch., *Alnus glutinosa* (L.) Gaertn., *Betula tianschanica* Rupr., *Dianthus capitatus* subsp. *andrzejowskianus* Zapał., *Drosera rotundifolia* L., *Chimaphila umbellata* (L.) W.P.C. Barton, *Lilium martagon* L., *Tulipa sylvestris* subsp. *australis* (Link) Pamp., *Tulipa suaveolens* Roth., *Nymphoides peltata* (S.G. Gmel.) Kuntze, *Dactylorhiza maculata* subsp. *fuchsii* (Druce) Hyl., *Epipactis palustris* (L.) Crantz., *Koeleria macrantha* subsp. *macrantha*, *Stipa pennata* L., *Adonis volgensis* Stev., *Pulsatilla patens* (L.) Mill., *Pulsatilla patens* subsp. *Flavescens* (Zucc.) Zämelis (= *Pulsatilla flavescens* (Zucc.) Juz.). Материалы собраны в результате обработки литературных источников и многочисленных ботанических экспедиций авторов по территории Костанайской области. Хранятся материалы в гербарном фонде Костанайского регионального университета имени Ахмет Байтурсынулы (акроним KSPI). Данная статья приводит конкретные данные о местонахождении эндемичных и редких видов на территории Костанайской области, что позволит эффективно защищать их и разработать дополнительные точечные меры охраны.

Ключевые слова: сведения о местонахождении, ревизия флоры, редкие виды растений, эндемичные виды растений, флора Республики Казахстан, флора Костанайской области, Красная книга РК, международные Гербарии

References

1 Pavlov, N. V. (Ed.). (1956–1964). *Flora Kazakhstana* [Flora of Kazakhstan]. Alma-Ata: Izdatelstvo Akademii Nauk KazSSR [in Russian].

2 Zaugolnova, L. B., & Pugachev, P. G. (1975). Flora i rastitelnost Naurzumskogo zapovednika [Flora and vegetation of the Naurzum Nature Reserve]. *Flora i rastitelnost Naurzumskogo gosudarstvennogo zapovednika — Flora and vegetation of the Naurzum State Nature Reserve*, 25–141. Kustanai [in Russian].

3 Perezhogin, Yu. V. (2013). Novye vidy tiulpanov iz Severnogo Kazakhstana [New tulip species from Northern Kazakhstan]. *Botanicheskii Zhurnal — Botanical Journal, 98*(12), 1558–1563 [in Russian].

4 Perezhogin, Yu. V. (2014). Validizatsiia dvukh nedeistvitelno obnarodovannykh nazvanii vidov *Tulipa* L. (Liliaceae) iz Severnogo Kazakhstana [Validation of two invalidly published species names of *Tulipa* L. (Liliaceae) from Northern Kazakhstan]. *Novosti Sistematiki Vysshikh Rastenii — News of Systematics of Higher Plants*, *45*, 145 [in Russian].

5 Perezhogin, Yu. V., Kulikov, P. V., & Kurlov, S. I. (2015). Dopolnenie k flore Kazakhstana [Supplement to the flora of Kazakhstan]. *Botanicheskii Zhurnal — Botanical Journal*, *100*(5), 501–503 [in Russian].

6 Perezhogin, Yu. V., & Kurlov, S. I. (2015). Dopolneniia i izmeneniia k spisku redkikh i ischezaiushchikh rastenii Kostanaiskoi oblasti (Severnyi Kazakhstan) [Additions and changes to the list of rare and endangered plants of the Kostanay Region (Northern Kazakhstan)]. *Vestnik Orenburgskogo Gosudarstvennogo Universiteta — Bulletin of Orenburg State University*, *10*(185), 43–47 [in Russian].

7 Perezhogin, Yu. V., & Kurlov, S. I. (2016). Dopolnenie k flore gosudarstvennogo prirodnogo rezervata «Altyn Dala» [Supplement to the flora of the Altyn Dala State Nature Reserve]. *Botanicheskii Zhurnal — Botanical Journal*, *101*(3), 302–308 [in Russian].

8 Perezhogin, Yu. V., & Kulikov, P. V. (2017). Novye vidy flory Kazakhstana [New species of the flora of Kazakhstan]. *Vestnik Orenburgskogo Gosudarstvennogo Universiteta — Bulletin of Orenburg State University*, *3*(203), 78–80 [in Russian].

9 Perezhogin, Yu. V. (2020). Floristicheskie nakhodki na territorii Kostanaiskoi oblasti [Floristic findings in the Kostanay Region]. *Vestnik Kostanaiskogo Gosudarstvennogo Pedagogicheskogo Instituta — Bulletin of Kostanay State Pedagogical Institute*, *2*(58), 73–78 [in Russian].

10 Perezhogin, Yu. V., & Erokhin, N. G. (2020). Floristicheskie nakhodki na territorii Kostanaiskoi oblasti (chast 2) [Floristic findings in the Kostanay Region (Part 2)]. *Vestnik Kostanaiskogo Gosudarstvennogo Pedagogicheskogo Instituta — Bulletin of Kostanay State Pedagogical Institute*, *3*(59), 35–40 [in Russian].

11 Perezhogin, Yu. V., Erokhin, N. G., & Baymangambetova, K.T. (2020). Floristicheskie nakhodki na territorii gosudarstvennogo prirodnogo rezervata «Altyn Dala» [Floristic findings in the "Altyn Dala" State Nature Reserve]. *Vestnik Kostanaiskogo Gosudarstvennogo Pedagogicheskogo Instituta — Bulletin of Kostanay State Pedagogical Institute*, *3*(59); 40–44 [in Russian].

12 Kubentayev, S. A., Alibekov, D. T., Perezhogin, Yu. V., Lazkov, G. A., Kupriyanov, A. N., Ebel, A. L., Izbastina, K. S., Borodulina, O. V., & Kubentayeva, B. B. (2024). Revised checklist of endemic vascular plants of Kazakhstan. *PhytoKeys*, 238, 241–279. https://doi.org/10.3897/phytokeys.238.114475

13 Seregin, A. P. (Ed.). (2020). Kollektsiia "Gerbarii MGU" [Collection "Herbarium of Moscow State University"]. *Depozitarii zhivykh sistem "Noev Kovcheg" (napravlenie "Rasteniia") — Noah's Ark Living Systems Repository (Plants)*. Moscow: Moskovskii gosudarstvennyi universitet. Retrieved from https://plant.depo.msu.ru/module/collectionpublic? d=P&openparams=%5Bopen-id%3D1524305 %5D [in Russian].

14 (1999). International Plant Names Index (IPNI). *www.ipni.org.* Retrieved from https://www.ipni.org

15 (2001). Global Biodiversity Information Facility (GBIF). *www.gbif.org*. Retrieved from https://www.gbif.org

16 (2017). Plants of the World Online. *www.plantsoftheworldonline.org.* Retrieved from https://www.plantsoftheworldonline.org

17 Perezhogin, Yu. V., Borodulina, O. V., Kurlov, S. I., Kakimzhanova, A. A., Manabayeva, Sh. A., & Shevtsov, A. B. (2024). *Sosudistye rasteniia Kostanaiskoi oblasti (konspekt, analiz i DNK-barkodirovanie Flory): monografiia* [Vascular plants of the Kostanay Region (Synopsis, analysis and DNA barcoding of the flora): monograph]. Astana: Tsentr Elit [in Russian].

18 (2004). Kostanaiskaia oblast [Kostanay Region]. *ru.wikipedia.org*. Retrieved from https://ru.wikipedia.org/wiki/Костанайская_область [in Russian].

19 Golovanov, Ya. M. (n.d.). Izobrazhenie *Astragalus kustanaicus* Popov [Image of *Astragalus kustanaicus* Popov]. Retrieved from https://www.plantarium.ru/page/image/id/479834.html [in Russian].

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Molecular diagnostics of viruses in apple rootstocks using RT-qPCR

The development of intensive horticulture in Kazakhstan requires the use of virus-free planting material, particularly apple rootstocks (*Malus domestica*), free of viral and viroid infections. Latent forms of pathogens, which do not exhibit visible symptoms but are easily transmitted through vegetative propagation, are considered the most hazardous. The objective of this study was to evaluate the phytosanitary status of apple rootstocks used in nurseries in southern Kazakhstan by employing reverse transcription quantitative polymerase chain reaction (RT-qPCR). A total of 24 samples from field and laboratory collections were analyzed. RNA extraction was performed using the "PhytoSorb®" kit, and diagnostics were conducted on the Bio-Rad CFX96 platform using multiplex LETGEN test kits. Four pathogens were identified: *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple green crinkle associated virus* (AGCaV), and *Apple hammerhead viroid* (AHVd). The viruses ASPV and AGCaV were found to be the most prevalent, occurring in both field and laboratory samples. These findings confirm the widespread circulation of these pathogens within the apple rootstock propagation system. The results emphasize the need for the implementation of regular molecular diagnostics, sanitation programs, and certification measures aimed at preventing the spread of latent infections and ensuring the sustainable development of horticulture in the region.

Keywords: *Malus domestica*, rootstock, viruses, viroids, RT-qPCR diagnostics.

Introduction

Apple (*Malus*) is one of the leading fruit crops and is cultivated in many countries worldwide, including under the agroclimatic conditions of Kazakhstan, where it occupies a significant share in fruit and berry production. Due to the high consumer demand and nutritional value of its fruits, apple plays a key role in the horticultural industry of the country. However, the successful development of the sector largely depends on the use of healthy planting material, particularly high-quality and virus-free rootstocks [1].

The production of rootstocks and the development of nursery practices in Kazakhstan are accompanied by a number of challenges, the most significant of which is the spread of viral infections among planting material [2]. Apple is predominantly propagated vegetatively—through grafting, cutting, or micropropagation. While this method of propagation ensures the preservation of varietal characteristics, it also facilitates the transmission of viral diseases, many of which may remain latent for extended periods without exhibiting visible symptoms [3, 4].

Latent viruses of fruit crops pose a serious threat to both commercial orchards and nurseries. Diseases caused by these pathogens reduce yield, impair the commercial and organoleptic qualities of fruits, and disrupt the compatibility between rootstock and scion. The greatest danger lies in the fact that, in the absence of pronounced symptoms, infected mother plants continue to be used for propagation, thereby contributing to the further spread of the infection [5].

The most significant viruses affecting apple include *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple stem grooving virus* (ASGV), *Apple mosaic virus* (ApMV), *Apple green crinkle associated virus* (AGCaV), as well as the recently identified viruses ARWV-1, ARWV-2, ApNMV, and the viroid AHVd. The presence of these pathogens is particularly critical during micropropagation [6, 7].

To address these challenges, modern molecular biology techniques, particularly reverse transcription quantitative polymerase chain reaction (RT-qPCR), enable accurate and sensitive detection of viruses even at the early stages of infection.

In the context of Kazakhstan, where the establishment of intensive orchards is a pressing task, the use of virus-free rootstocks is of critical importance. In this regard, the relevance of diagnosing and monitoring viral infections in nursery material is increasing. The application of molecular genetic techniques, such as reverse transcription quantitative polymerase chain reaction (RT-qPCR), enables highly accurate detection of viral pathogens even in asymptomatic plants [8]. This, in turn, provides an opportunity to establish a healthy foundation for the development of modern orchards and to enhance the efficiency of fruit and berry production in the country. The implementation of such approaches opens prospects for creating highly productive orchards resistant to viral degradation and contributes to the sustainable development of the industry at the national level [9].

The present study is aimed at identifying viruses and viroids of apple rootstocks circulating within the nursery production system of Kazakhstan, which will provide a basis for the implementation of phytosanitary control measures. The objective of the research is to assess the phytosanitary status of apple rootstocks used in Kazakhstani nurseries and to determine the spectrum of viral and viroid pathogens through the application of reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Experimental

Objects of the Study. The study was conducted on 24 samples of apple rootstocks (*Malus domestica Borkh.*) collected from various sources, including a nursery farm in the Almaty region and the collection of basic plants at the Kazakh Research Institute of Fruit and Vegetable Growing. Sample collection was carried out during the vegetation season (March–September 2024-2025).

Plant Material Collection. Each sample represented a single tree, from which 15–20 fully developed leaves were collected in four directions (east, west, north, south). Field monitoring included a visual assessment of symptoms, while in vitro samples showed no visible signs of infection. Leaves were stored at +4 °C until RNA extraction. Sampling was carried out in accordance with EPPO standards [10]. All samples were tested for the presence of viruses according to EPPO standards, as well as for newly widespread viruses and viroids, including ASPV, ACLSV, ApMV, ASGV, ARWV1, ApNMV, AGCaV, and AHVd.

RNA Extraction. RNA was extracted from leaves using the PhytoSorb® kit (Syntol LLC) following a modified protocol by Mekuria et al. [11]. Leaf tissue (200 mg) was ground in liquid nitrogen and extracted with a buffer containing 4.4 % PVP-40 and 1 % sodium metabisulfite, followed by vortex mixing. The quality and concentration of RNA were determined using a NanoDrop 1000 spectrophotometer. Purity was assessed by the absorbance ratios at 280/260 and 260/230 nm (1.8–2.3). Samples were stored at −20 °C.

RT-qPCR. Detection of viruses (ACLSV, ASPV, ApMV, ASGV, ARWV-1, ARWV-2, ApNMV, AGCaV) and the viroid AHVd was carried out using RT-qPCR with LETGEN® (TaqMan®) kits and the Bio-Rad CFX96 platform. Each reaction was performed in triplicate. The use of certified reagents and equipment ensured high sensitivity and eliminated false-positive results.

Throughout the study, the highly sensitive Bio-Rad CFX96 system and certified TaqMan-based kits were employed, providing high analytical specificity and preventing false-positive outcomes (Table 1).

Table 1

Sampling sites and names of plant material used for virus diagnostics

Location of samples	№	Name of samples
Nursery Farm, **Enbekshikazakh district, Almaty region**	1	M-9
	2	M-7
	3	MM-106
	4	B-9
Collection of basic plants under protected conditions, Department of Biotechnology of Horticultural Crops, Kazakh Research Institute of Fruit and Vegetable Growing, Almaty	5	Б 16-20
	6	Б 7-35
	7	62-396
	8	APM-18
	9	Zhetysu 5

Continuation of Table 1

Location of samples	№	Name of samples
Collection of basic plants under protected conditions, Talgar district, Almaty region, Almalyk village, Talgar Research Facility, Kazakh Research Institute of Fruit and Vegetable Growing	10	APM-18
Field collection, Talgar district, Almaty region, Almalyk village, Talgar Research Facility, Kazakh Research Institute of Fruit and Vegetable Growing	11	Б-7-35
	12	Б-16-20
	13	6-4-8
	14	Zhetysu 3
	15	Zhetysu 4
	16	Zhetysu 5
	17	Zhetysu 6
	18	Zhetysu 7
	19	Б-7-35
	20	Б-16-20
	21	Zhetysu 2
	22	Б-7-35 (M)
	23	Б-16-20 (M)
	24	62-396 (M)

Results and Discussion

The study included field and laboratory examinations of 24 samples of apple rootstocks (*Malus domestica* Borkh.) collected from various sources: a nursery farm in the Almaty region, the collection of basic plants under protected conditions, and the field collection of the Kazakh Research Institute of Fruit and Vegetable Growing. The results are presented using the following symbols: "+" indicates that a virus or viroid was detected in the sample, while "–" indicates its absence (Table 2).

Table 2

Name, origin, and results of virus detection in the studied apple rootstocks

Location of samples	Name of samples	Name of viruses			
		ACLSV	***ASPV***	***AGCaV***	***AHVd***
Nursery Farm, Enbekshikazakh district, Almaty region	M-9	+	+	–	–
	M-7	–	–	–	+
	MM-106	–	–	–	–
	B-9	–	–	–	–
Collection of basic plants under protected conditions, Department of Biotechnology of Horticultural Crops, Kazakh Research Institute of Fruit and Vegetable Growing, Almaty	B 16-20	+	–	+	–
	B 7-35	–	–	+	–
	62-396	–	–	–	–
	APM-18	–	–	–	–
	Zhetysu 5	–	–	–	–
Collection of basic plants under protected conditions, Talgar district, Almaty region, Almalyk village, Talgar Research Facility, Kazakh Research Institute of Fruit and Vegetable Growing	APM-18	–	–	–	–

Continuation of Table 2

Location of samples	Name of samples	Name of viruses			
		ACLSV	***ASPV***	***AGCaV***	***AHVd***
Field collection, Talgar district, Almaty region, Almalyk village, Talgar Research Facility, Kazakh Research Institute of Fruit and Vegetable Growing	B-7-35	–	–	+	–
	B-16-20	–	–	+	–
	6-4-8	–	+	+	–
	Zhetysu 3	–	–	+	–
	Zhetysu 4	–	–	–	–
	Zhetysu 5	–	–	+	–
	Zhetysu 6	–	–	+	–
	Zhetysu 7	–	–	–	–
	B-7-35	+	+	+	–
	B-16-20	+	+	+	–
	Zhetysu 2	–	–	+	–
	B-7-35 (M)	+	+	–	–
	B-16-20 (M)	–	+	–	–
	62-396 (M)	–	–	+	–

Diagnostics performed using the RT-qPCR method revealed positive results for three viruses—ACLSV, ASPV, and AGCaV—as well as for the viroid AHVd (Table 2). The remaining viruses (ApMV, ASGV, ApNMV, ARWV-1, ARWV-2) were not detected in any of the analyzed samples (Fig. 1).

ACLSV was detected in five samples: M-9 (nursery farm), B-16-20 (collection of basic plants under protected conditions, field collection of the Kazakh Research Institute of Fruit and Vegetable Growing), as well as its clone B-16-20 (M), and B-7-35 (field plot of the Kazakh Research Institute of Fruit and Vegetable Growing) along with its clone B-7-35 (M). The amplification curves exhibited signal enhancement at early cycles (Ct < 30), confirming a high viral load and the reliable presence of ACLSV.

ASPV was detected in six samples: M-9, 6-4-8, B-7-35, B-16-20 (field site), as well as in clonal forms B-7-35 (M) and B-16-20 (M). This virus exhibited a broader distribution compared to ACLSV. The recorded Ct values ranged from 20 to 35, indicating the presence of both latent and active forms of infection.

AGCaV was the most frequently detected pathogen, identified in 12 samples. It was found in the rootstocks B-16-20, B-7-35, 6-4-8, as well as in cultivars from the Zhetysu collection (Zhetysu 2, 3, 5, 6) and in the samples 62-396 (M) and Zhetysu-5. Its occurrence in materials from various sources indicates its active circulation. Such prevalence highlights the ongoing circulation of AGCaV within the nursery propagation system.

The viroid AHVd was detected in only one sample (M-7) originating from the nursery farm. Signal amplification was observed after cycle 39, exceeding the recommended Ct threshold of ≤35. Nevertheless, the presence of AHVd in one of the rootstocks warrants additional monitoring, given its potential phytopathogenicity.

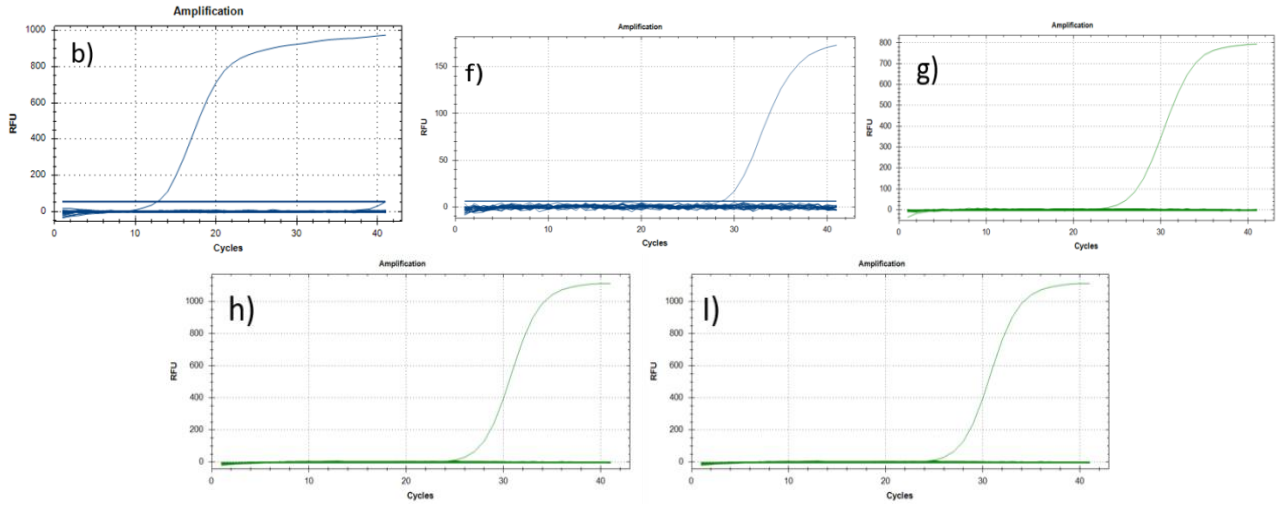

Figure 1. Negative amplification results obtained by RT-qPCR
b) ApMV; f) ASGV; g) ApNMV; h) ARWV-1; i) ARWV-2

This study was aimed at identifying viral pathogens in mother plant blocks and assessing the phytosanitary status of apple rootstocks. To date, field collections of pome crops in Kazakhstan have not undergone systematic virus testing, making the obtained results significant for developing strategies for sanitation and preventing the further spread of infectious agents in nursery production.

Monitoring, including visual inspection, molecular diagnostics [12], and selection of resistant genotypes [13], forms the basis of orchard protection. Since viral infections cannot be controlled with fungicides, the use of certified virus-free planting material remains the key preventive measure [14].

Particular attention should be given to AGCaV, which demonstrated the highest prevalence in both field and greenhouse samples. This virus is associated with fruit deformation, characterized by deep depressions, cracks, brown spots, and a reduction in tree productivity, in some cases leading to severe decline [15]. ASPV was also frequently detected, including asymptomatic carriage in clonal rootstocks. It induces pitting in the wood of certain cultivars (e.g., *Charden* and *Virginia Crab*), but often remains symptomless in commercial cultivars [16]. ACLSV negatively affects tree growth, yield, and fruit quality. In susceptible rootstocks (e.g., *Asami*), it triggers a hypersensitive reaction at the graft union, resulting in plant death—known as "topworking disease" [17]. Even in the absence of visible symptoms, infected plants exhibit impaired photosynthetic processes and reduced physiological potential. AHVd is considered pathogenic, as it has been associated with trunk cracking, necrosis, shoot weakening, and growth retardation across different continents [18]. Recently, AHVd was confirmed to be a true viroid capable of autonomous replication and inducing cellular disorders [19].

Our findings confirm that even latent infections caused by these pathogens can significantly affect the quality and viability of rootstocks. The detection of ACLSV, ASPV, AGCaV, and AHVd in symptomless samples underscores the necessity of routine RT-qPCR diagnostics and the implementation of sanitation programs for rootstock material [20, 21]. These results highlight the importance of an integrated approach to viral phytosanitary security. In particular, the application of *in vitro* elimination techniques, such as thermotherapy and shoot tip culture, can be recommended, as these methods have previously demonstrated their effectiveness in controlling PPV and ACLSV in stone fruit crops [22]. Therefore, the results of this study contribute to the development of a comprehensive strategy for virological control, integrating molecular, biotechnological, and breeding approaches to support sustainable nursery production in Kazakhstan.

Conclusions

The aim of this study was to assess the phytosanitary status of apple rootstocks used in nursery production in Kazakhstan and to identify the spectrum of viral and viroid pathogens. The results demonstrated the presence of both widespread and newly detected pathogens in collection mother blocks and nurseries. Furthermore, these findings emphasize the necessity of routine molecular diagnostics for the efficient detection and management of viruses, particularly within breeding and certification programs. Future research should focus on developing comprehensive strategies to control viruses and viroids within mother plant and nursery systems.

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Conflict of interest

The authors declare no conflict of interest.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Askarova M.A.** — Data curation, Writing — original draft, Writing — review & editing; **Yusupova Z.YA.** — Investigation, Methodology; **Madenova A.K.** — **Resources,** Validation; **Abdikerimova R.A.** — Formal analysis, Investigation, Methodology; **Kabylbekova B.Zh.** — Project administration, Supervision, Validation, Visualization.

References

1 Ромаданова Н. В. Биотехнология получения безвирусных саженцев яблони [Электронный ресурс] / Н. В. Ромаданова, С. В. Кушнаренко // Вестник Карагандинского университета. Серия: Биология. Медицина. География. — 2021. — №3(103). — С. 102–118. — Режим доступа: https://feb.buketov.edu.kz/bmg-vestnik/issue/view/46/46.

2 Кабылбекова Б. Ж. Определение состава вирусных болезней клоновых подвоев косточковых культур в Казахстане и особенности размножения *in vitro* здоровых растений [Электронный ресурс] / Б. Ж. Кабылбекова, Т. Нурсейтова, З. Юсупова, И. Ковальчук, Н. Чуканова, М. Уразаева, А.К. Маденова // Ізденістер, нәтижелер — Исследования, результаты. — 2024. — № 3(103). — С. 86–97. — Режим доступа: https://journal.kaznaru.edu.kz/index.php/research/article/view/696.

3 Kerimbek N. Mapping the distribution of viruses in wild apple populations in the southeast region of Kazakhstan / N. Kerimbek, M. Khusnitdinova, A. Taskuzhina, A. Kapytina, A. Pozharsky, A. Sagitov, D. Gritsenko // Forests. — 2025. — Vol. 16, No. 7. — P. 1119. DOI: https://doi.org/10.3390/f16071119.

4 Kerimbek N. M. Assessment of occurrence and diversity of apple viruses in South Kazakhstan [Electronic resource] / N. M. Kerimbek, A. I. Kapytina, M. A. Khusnitdinova, A. K. Taskuzhina // Science and education. — 2024. — Vol. 2, No 2(75). — P. 79–85. — Access mode: https://rep.wkau.kz/server/api/core/bitstreams/36844a1f-ec1d-404e-b651-3189a8ffa7bd/content

5 Kairova A. Zh. Assessment of apple (*Malus domestica* Borkh.) rootstocks for resistance to fire blight (*Erwinia amylovora*) in Southern Kazakhstan / A. Zh. Kairova, G. M. Baymoldina, A. B. Sharipova, Zh. T. Argyngaliyeva, A. S. Shaimardanova, A. K. Akhmetova, E.A. Kairov // Applied Sciences. — 2023. — Vol. 13(20). — P. 11530. DOI: https://doi.org/10.3390/app132011530.

6 Wunsch A. Viruses of apple are seedborne but likely not vertically transmitted / A. Wunsch, B. Hoff, M. Miranda Sazo, J. van Zoeren, K. H. Lamour, O. P. Hurtado Gonzales, M. Fuchs // Viruses. — 2024. — Vol. 16(1). — P. 95. DOI: https://doi.org/10.3390/v16010095.

7 Ben Mansour K. High throughput sequencing reveals apple virome diversity and novel viruses in the Czech Republic / K. Ben Mansour, I. Koloniuk, J. Brožová, M. Komínková, J. Přibylová, T. Sarkisova, J. Sedlák, J. Špak, P. Komínek // Viruses. — 2025. — Vol. 17(5). — P. 650. DOI: https://doi.org/10.3390/v17050650.

8 Omasheva M. Y. SSR genotyping of Kazakhstani apple varieties: Identification of alleles associated with resistance to highly destructive pathogens [Electronic resource] / M. Y. Omasheva, A. Pozharskiy, A. Maulenbay, N. A. Ryabushkina // Biotechnology: Theory and Practice. — 2016. — P. 350–356. — Access mode: https://biotechlink.org/index.php/journal/article/view/168.

9 Gritsenko D. Simultaneous detection of five apple viruses by RT-PCR / D. Gritsenko, K. Aubakirova, I. Voitsekhovskiy, N. Galiakparov // International Journal of Biology and Chemistry. — 2020. — Vol. 13(1). DOI: https://doi.org/10.26577/ijbch.2020.v13.i1.13.

10 OEPP/EPPO. Certification scheme for pathogen-tested material of *Malus*, *Pyrus* and *Cydonia* (Standard PM 4/27(1)) [Electronic resource]. — Paris: European and Mediterranean Plant Protection Organization, 1999. — Access mode: https://gd.eppo.int/standards/PM4/PM4-27-1.

11 Mekuria G. Comparison of ELISA and RT-PCR for the detection of Prunus necrotic ring spot virus and Prune dwarf virus in almond (Prunus dulcis) [Electronic resource] / G. Mekuria, S. A. Rhamesh, E. Alberts, T. Bertozzi, M. Wirthensohn, G. Collins, M. Sedgley // Journal of Virological Methods. — 2003. — Vol. 114. — P. 65–69. — Access mode: https://pubmed.ncbi.nlm.nih.gov/14599680.

12 Galymbek K. Phytosanitary diagnosis of apple fungal diseases in Almaty region / K. Galymbek, A. Madenova, S. Bakirov // Research on Crops. — 2024. — Vol. 25. — P. 499–508. DOI: http://dx.doi.org/10.31830/2348-7542.2024.ROC-1112.

13 Fazio G. Virus studies in the Geneva® apple rootstock breeding program [Electronic resource] / G. Fazio, J. C. Bettoni, L.C. Costa, O. P. Hurtado-Gonzales, M. Al Rwahnih, A. Steinberger, T. Robinson // New York Fruit Quarterly. — 2022. — P. 20–24. — Access mode: https://www.researchgate.net/publication/362123931.

14 James D. Perspective on strategies for controlling the spread of *Plum pox virus*, causal agent of sharka/plum pox disease / D. James // III International Symposium on *Plum Pox Virus*. — 2016. — Vol. 1163. — P. 129–136. DOI: https://doi.org/10.17660/ActaHortic.2017.1163.19.

15 Manzoor S. Unveiling viral threats to temperate pome fruits: Characterization, transmission, and sustainable management strategies / S. Manzoor, S. U. Nabi, A. Ayaz, B. Rasool, S. K. Sharma, M. H. Chesti, M. F. Rabbee // Frontiers in Microbiology. — 2025. — Vol. 16. — P. 1560720. DOI: https://doi.org/10.3389/fmicb.2025.1560720.

16 Wright A. A bushel of viruses: Identification of seventeen novel putative viruses by RNA seq in six apple trees / A. A. Wright, A. R. Cross, S. J. Harper // PLoS ONE. — 2020. — Vol. 15(1). — e0227669. DOI: https://doi.org/10.1371/journal.pone.0227669.

17 Pedrelli A. Apple chlorotic leaf spot virus infection affects the physiology and biochemistry of leaves and the quality of fruits of Tuscany (Italy) autochthonous apple varieties / A. Pedrelli, C. Nali, A. Panattoni, E. Pellegrini, L. Cotrozzi // Plant Pathology. — 2025. — Vol. 74(1). DOI: https://doi.org/10.1111/ppa.14004.

18 Serra P. Apple hammerhead viroid-like RNA is a bona fide viroid: Autonomous replication and structural features support its inclusion as a new member in the genus Pelamoviroid / P. Serra, A. Messmer, D. Sanderson, D. James. R. Flores // Virus Research. — 2018. — Vol. 249. — P. 8–15. DOI: https://doi.org/10.1016/J.VIRUSRES.2018.03.001.

19 Sedlák J. Elimination of Solanum nigrum ilarvirus 1 and apple hammerhead viroid from apple cultivars using antivirals ribavirin, rimantadine, and zidovudine / J. Sedlák, J. Přibylová, I. Koloňuk, J. Špak, O. Lenz, M. Semerák // Viruses. — 2023. — Vol. 15(8). — P. 1684. DOI: https://doi.org/10.3390/v15081684.

20 Маденова А. К. Идентификация вирусных заболеваний яблони в казахстанских садах методом ОТ-ПЦР-РВ / А. К. Маденова, Д. И. Калдыбаева, Р. А. Әбдікәрімова, А.А. Қуан // Ізденістер, нәтижелер — Исследования, результаты. — 2024. — № 3(103). — С. 97–107. DOI: https://doi.org/10.37884/3-2024/11.

21 Guo X. Identification and analysis of apple virus diseases based on virome sequencing technology / X. Guo, S. Huang, S. Wu, Y. Liu, M. Wang, C. Song, T. Bai, S. Song, H. Pang, C. Jiao, S. Zheng // Acta Horticulturae Sinica. — 2022. — Vol. 49(7). — P. 1415–1428. DOI: https://doi.org/10.16420/j.issn.0513-353x.2021-0450.

22 Kabylbekova B. Application of In Vitro Techniques for Elimination of Plum Pox Virus (PPV) and Apple Chlorotic Leaf Spot Virus (ACLSV) in Stone Fruits / B. Kabylbekova, T. Nurseitova, Z. Yussupova, T. Turdiyev, I. Kovalchuk, S. Dolgikh, S. Soltanbekov, A. Seisenova, A. Madenova // Horticulturae. — 2025. — Vol. 11(6). — P. 633. DOI: https://doi.org/10.3390/horticulturae11060633.

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Алма телітушілеріндегі вирустық инфекцияларды RT-qPCR әдісімен анықтау

Қазақстанда интенсивті бақ шаруашылығын дамыту үшін, әсіресе вирус және вироид инфекцияларынан таза, сауықтырылған отырғызу материалын, соның ішінде алманың телітушілерін (*Malus domestica*) қолдану қажет. Ең қауіптілері — айқын белгілері жоқ, бірақ вегетативтік көбейту кезінде оңай таралатын латентті патогендер. Зерттеудің мақсаты — Қазақстанның оңтүстігінде тәлімбақтарда қолданылатын алма телітушілерінің фитосанитарлық жағдайын нақты уақыт режиміндегі кері транскрипциялы полимеразды тізбекті реакция (RT-qPCR) әдісімен бағалау. Талдау үшін далалық және зертханалық коллекциялардан 24 үлгі алынды. РНҚ бөліп алу «ФитоСорб®» жинағының көмегімен жүргізілді, ал диагностика Bio-Rad CFX96 платформасында LETGEN мультиплекстік тест-жүйелері арқылы орындалды. Нәтижесінде төрт патоген анықталды: *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple green crinkle associated virus* (AGCaV) және *Apple hammerhead viroid* (AHVd) вироиды. Ең жиі кездескен патогендер ASPV және AGCaV вирустары, олар әрі далалық, әрі зертханалық үлгілерде тіркелді. Алынған деректер алма телітушілерін көбейту жүйесінде осы патогендердің жоғары деңгейде таралғанын көрсетті. Зерттеу нәтижелері жасырын инфекциялардың таралуын болдырмау және аймақтағы бақ шаруашылығының тұрақты дамуын қамтамасыз ету үшін тұрақты молекулалық диагностика, сауықтыру және сертификаттау бағдарламаларын енгізудің маңыздылығын дәлелдейді.

Кілт сөздер: Malus domestica, телітуші, вирус, вироид, RT-qPCR анықтау

М.А. Асқарова, З.Я. Юсупова, А.К. Маденова, Р.А. Абдикаримова, Б.Ж. Кабылбекова

Молекулярная диагностика вирусов на подвоях яблони методом RT-qPCR

Развитие интенсивного садоводства в Казахстане требует применения оздоровлённого посадочного материала, особенно подвоев яблони (*Malus domestica*), свободных от вирусных и вироидных инфекций. Наиболее опасными считаются латентные формы патогенов, не имеющие выраженных симптомов, но легко передающиеся при вегетативном размножении. Цель настоящего исследования заключалась в оценке фитосанитарного состояния подвоев яблони, используемых в питомниководстве юга

Казахстана, с применением метода полимеразной цепной реакции с обратной транскрипцией в реальном времени (RT-qPCR). Для анализа было отобрано 24 образца из полевых и лабораторных коллекций. Выделение РНК проводилось с использованием набора «ФитоСорб®», а диагностика — на платформе Bio-Rad CFX96 с использованием мультиплексных тест-систем LETGEN. В результате были выявлены четыре патогена: *Apple chlorotic leaf spot virus* (ACLSV), *Apple stem pitting virus* (ASPV), *Apple green crinkle associated virus* (AGCaV) и вироид *Apple hammerhead viroid* (AHVd). Наиболее широкое распространение показали вирусы ASPV и AGCaV, встречающиеся как в полевых, так и в лабораторных образцах. Данные подтверждают высокую циркуляцию этих патогенов в системе размножения подвоев яблони. Результаты подчёркивают необходимость внедрения регулярной молекулярной диагностики, программ оздоровления и сертификации, направленных на предотвращение распространения скрытых инфекций и обеспечение устойчивого развития садоводства в регионе.

Ключевые слова: Malus domestica, подвой, вирусы, вироиды, RT-qPCR диагностика

References

1 Romadanova, N. V., & Kushnarenko, S. V. (2021). Biotekhnologiia polucheniia bezvirusnykh sazhentsev [Biotechnology for Producing Virus-Free Apple Seedlings]. *Vestnik Karagandinskogo universiteta. Seriia: Biologiia. Meditsina — Bulletin of Karaganda University. Biology. Medicine. Geography Series*, *3*(103), 102–118. Retrieved from https://feb.buketov.edu.kz/bmg-vestnik/issue/view/46/46 [in Russian].

2 Kabylbekova, B. Zh., Nurseitova, T., Yusupova, Z., Kovalchuk, I., Chukanova, N., Urazaeva, M., & Madenova, A. K. (2024). Opredelenie sostava virusnykh boleznei klonovykh podvoev kostochkovykh kultur v Kazakhstane i osobennosti razmnozheniia *in vitro* zdorovykh rastenii [Determination of viral diseases composition of stone fruit clonal rootstocks in Kazakhstan and *in vitro* reproduction features of healthy plants]. *Izdenister, Natizheler — Issledovaniia, Rezultaty — Research and Results*, *3*(103), 86–97. Retrieved from https://journal.kaznaru.edu.kz/index.php/research/article/view/696 [in Russian].

3 Kerimbek, N., Khusnitdinova, M., Taskuzhina, A., Kapytina, A., Pozharsky, A., Sagitov, A., & Gritsenko, D. (2025). Mapping the distribution of viruses in wild apple populations in the southeast region of Kazakhstan. *Forests, 16*(7), 1119. https://doi.org/10.3390/f16071119

4 Kerimbek, N., Kapytina, A. I., Khusnitdinova, M. A., & Taskuzhina, A. K. (2024). Assessment of occurrence and diversity of apple viruses in South Kazakhstan. *Science and Education, 2*, 2(75), 79–87. Retrieved from https://www.researchgate.net/publication/391587146

5 Kairova, A. Zh., Baymoldina, G. M., Sharipova, A. B., Argyngaliyeva, Zh. T., Shaimardanova, A. S., Akhmetova, A. K., & Kairov, E. A. (2023). Assessment of apple (*Malus domestica* Borkh.) rootstocks for resistance to fire blight (*Erwinia amylovora*) in Southern Kazakhstan. *Applied Sciences, 13*(20), 11530. https://doi.org/10.3390/app132011530

6 Wunsch, A., Hoff, B., Miranda Sazo, M., van Zoeren, J., Lamour, K. H., Hurtado Gonzales, O. P., & Fuchs, M. (2024). Viruses of apple are seedborne but likely not vertically transmitted. *Viruses, 16*(1), 95. https://doi.org/10.3390/v16010095

7 Ben Mansour, K., Koloniuk, I., Brožová, J., Komínková, M., Přibylová, J., Sarkisova, T., Sedlák, J., Špak, J., & Komínek, P. (2025). High throughput sequencing reveals apple virome diversity and novel viruses in the Czech Republic. *Viruses, 17*(5), 650. https://doi.org/10.3390/v17050650

8 Omasheva, M. Y., Pozharskiy, A., Maulenbay, A., & Ryabushkina, N. A. (2016). SSR genotyping of Kazakhstani apple varieties: Identification of alleles associated with resistance to highly destructive pathogens. *Biotechnology: Theory and Practice*, 350–356. Retrieved from https://biotechlink.org/index.php/journal/article/view/168

9 Gritsenko, D., Aubakirova, K., Voitsekhovskiy, I., & Galiakparov, N. (2020). Simultaneous detection of five apple viruses by RT-PCR. *International Journal of Biology and Chemistry, 13*(1). https://doi.org/10.26577/ijbch.2020.v13.i1.13

10 OEPP/EPPO (1999). Certification scheme for pathogen-tested material of *Malus, Pyrus* and *Cydonia* (Standard PM 4/27(1)). Paris: European and Mediterranean Plant Protection Organization. Retrieved from https://gd.eppo.int/standards/PM4/PM4-27-1

11 Mekuria, G., Rhamesh, S. A., Alberts, E., Bertozzi, T., Wirthensohn, M., Collins, G., & Sedgley, M. (2003). Comparison of ELISA and RT-PCR for the detection of Prunus necrotic ring spot virus and Prune dwarf virus in almond (Prunus dulcis). *Journal of Virological Methods, 114*, 65–69. Retrieved from https://pubmed.ncbi.nlm.nih.gov/14599680

12 Galymbek, K., Madenova, A., & Bakirov, S. (2024). Phytosanitary diagnosis of apple fungal diseases in Almaty region. *Research on Crops, 25*, 499–508. http://dx.doi.org/10.31830/2348-7542.2024.ROC-1112

13 Fazio, G., Bettoni, J. C., Costa, L. C., Hurtado-Gonzales, O. P., Al Rwahnih, M., Steinberger, A., & Robinson, T. (2022). Virus studies in the Geneva® apple rootstock breeding program. *New York Fruit Quarterly*, 20–24. Retrieved from https://www.researchgate.net/publication/362123931

14 James, D. (2016). Perspective on strategies for controlling the spread of *Plum pox virus*, causal agent of sharka/plum pox disease. In *III International Symposium on Plum Pox Virus, 1163*, 129–136. https://doi.org/10.17660/ActaHortic.2017.1163.19

15 Manzoor, S., Nabi, S. U., Ayaz, A., Rasool, B., Sharma, S. K., Chesti, M. H., & Rabbee, M. F. (2025). Unveiling viral threats to temperate pome fruits: Characterization, transmission, and sustainable management strategies. *Frontiers in Microbiology, 16*, 1560720. https://doi.org/10.3389/fmicb.2025.1560720

16 Wright, A. A., Cross, A. R., & Harper, S. J. (2020). A bushel of viruses: Identification of seventeen novel putative viruses by RNA seq in six apple trees. *PLoS ONE, 15*(1), e0227669. https://doi.org/10.1371/journal.pone.0227669

17 Pedrelli, A., Nali, C., Panattoni, A., Pellegrini, E., & Cotrozzi, L. (2025). Apple chlorotic leaf spot virus infection affects the physiology and biochemistry of leaves and the quality of fruits of Tuscany (Italy) autochthonous apple varieties. *Plant Pathology, 74*(1), https://doi.org/10.1111/ppa.14004

18 Serra, P., Messmer, A., Sanderson, D., James, D., & Flores, R. (2018). Apple hammerhead viroid-like RNA is a bona fide viroid: Autonomous replication and structural features support its inclusion as a new member in the genus *Pelamoviroid*. *Virus Research, 249*, 8–15. https://doi.org/10.1016/J.VIRUSRES.2018.03.001

19 Sedlák, J., Přibylová, J., Koloňuk, I., Špak, J., Lenz, O., & Semerák, M. (2023). Elimination of *Solanum nigrum ilarvirus 1* and apple hammerhead viroid from apple cultivars using antivirals ribavirin, rimantadine, and zidovudine. *Viruses, 15*(8), 1684. https://doi.org/10.3390/v15081684

20 Madenova, A. K., Kaldybayeva, D. I., Abdikarimova, R. A., & Kuan, A. A. (2024). Identifikatsiia virusnykh zabolevanii yabloni v kazakhstanskikh sadakh metodom OT-PTsR-RV [Identification of apple viral diseases in Kazakhstani orchards by RT-qPCR method]. *Izdenister, Natizheler — Issledovaniia, Rezultaty — Research and Results*, 3(103), 97–107. https://doi.org/10.37884/3-2024/11 [in Russian].

21 Guo, X., Huang, S., Wu, S., Liu, Y., Wang, M., Song, C., Bai, T., Song, S., Pang, H., Jiao, C., & Zheng, S. (2022). Identification and analysis of apple virus diseases based on virome sequencing technology. *Acta Horticulturae Sinica, 49*(7), 1415–1428. https://doi.org/10.16420/j.issn.0513-353x.2021-0450

22 Kabylbekova, B., Nurseitova, T., Yussupova, Z., Turdiyev, T., Kovalchuk, I., Dolgikh, S., Soltanbekov, S., Seisenova, A., & Madenova, A. (2025). Application of In Vitro Techniques for Elimination of Plum Pox Virus (PPV) and Apple Chlorotic Leaf Spot Virus (ACLSV) in Stone Fruits. *Horticulturae, 11*(6), 633. https://doi.org/10.3390/horticulturae11060633

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Design of primers and probes for detection of influenza A and SARS-CoV-2 viruses RNA by real-time RT-PCR

Cost-effective, accurate, and rapid analysis is essential for testing and diagnosing both common and emerging viruses in clinical virology laboratories. In this study, we designed and selected oligonucleotides for the detection of influenza A virus and SARS-CoV-2 using real-time reverse transcription PCR (RT-qPCR). The development of domestic test systems for diagnosing influenza A virus and SARS-CoV-2 is an urgent task due to the need for early disease detection. The aim of this study is to select primers and probes for the diagnosis of influenza A and SARS-CoV-2 using reverse transcription PCR in real-time (RT-PCR RT). We present the results of designing primers and probes for the identification of influenza A and SARS-CoV-2 RNA. In our studies on the selection of specific primers and probes, the M gene was chosen as a target gene for detecting the influenza A virus, and the RdRp gene for the SARS-CoV-2 virus. A pair of oligonucleotide primers was selected and synthesized for influenza A InfM2 F and InfM2 R, as well as the InfM2 Probe, and for SARS-CoV-2 — RdRp-1 F and RdRp-2 R, the RdRp-2 Probe, which, when performing RT-PCR RT with a working concentration of 20 pmol showed high efficiency in detecting the influenza A virus and SARS-CoV-2. Primers were selected using the Primer Blast and Vector NTI computer programs. The designed primers and probes will be further used to create a domestic multiplex RT-PCR RT test system.

Keywords: influenza A, coronavirus, RT-PCR, diagnostics, test-system.

Introduction

Acute respiratory infections (ARIs) are a broad group of acute infectious diseases caused by various pathogens, such as viruses, bacteria, chlamydia, and mycoplasma [1]. ARIs pose a serious threat to humanity, directly affecting the daily lives of millions of people and negatively impacting the global economy [2, 3, 4]. Currently, respiratory infections account for up to 90 % of all infectious diseases [5]. Each year, over 1 billion cases of acute respiratory infections are recorded worldwide, which significantly exceeds the number of patients with serious diseases such as cancer, HIV, coronary heart disease, or malaria [1].

Influenza occupies a significant place among acute respiratory infections, remaining one of the most significant viral infections. Approximately 1 billion cases of seasonal influenza are registered annually, of which 3 to 5 million are severe. Respiratory diseases caused by influenza viruses kill between 290 000 and 650 000 people annually [6].

There are four types of seasonal influenza viruses: types A, B, C and D. Influenza A and B viruses circulate and cause seasonal epidemics. Influenza A viruses are divided into subtypes based on the protein combinations on the virus surface. Currently, influenza viruses of subtypes A(H1N1) and A(H3N2) circulate among humans. A(H1N1) is also referred to as A(H1N1)pdm09 because it caused the 2009 pandemic and replaced the seasonal influenza A(H1N1) virus that circulated before 2009. Only influenza type A viruses are known to cause pandemics.

Recently, the emergence and rapid spread of a novel coronavirus (SARS-CoV-2), which has become a significant acute respiratory disease, has raised serious concerns about global health [7]. Since its discovery, SARS-CoV-2 has rapidly spread to more than 230 countries. As of January 2025, more than 777 million confirmed cases of infection have been recorded worldwide, resulting in 7 079 925 deaths [8].

The clinical manifestations of infection caused by SARS-CoV-2 are similar to those that occur with influenza virus infection [9]. Highly effective and sensitive diagnostic tests are needed to accurately distinguish influenza viruses from SARS-CoV-2 [10]. Differential diagnosis of SARS-CoV-2 and influenza viruses is essential for the development of effective public health strategies and patient treatment. This is especially relevant for identifying suspected cases, severe forms of the disease, and assessing potential outbreak threats. Integrating influenza testing into existing COVID-19 assays significantly reduces the time required for diagnosis and improves the use of existing equipment, resources and personnel. This approach is cost-effective and contributes to a more effective fight against COVID-19 and influenza [11].

Research into the development and application of rapid diagnostic methods for influenza A and SARS-CoV-2 viruses based on real-time RT-PCR is ongoing and is being conducted in many countries worldwide [12–15]. The development of a domestic test-system for the diagnosis of influenza A and SARS-CoV-2 viruses is driven by the need for early detection. The advantages of real-time RT-PCR test-systems include their high sensitivity and specificity. This is achieved through the use of species-specific primer sets and fluorescent probes, which, combined with specially selected reaction conditions, enable the detection of trace amounts of microbial RNA molecules in the analyzed sample.

Influenza A virus is one of the most widespread and variable viruses causing epidemics and pandemics. The viral genome consists of eight segments of negative-sense single-stranded RNA. Direct detection of viral RNA avoids errors associated with antigenic variability and cross-reactions in immunodiagnostic tests [16, 17]. When developing a test-system, it is important to carefully select primers and probes to ensure maximum specificity and sensitivity. The most commonly used primer targets are the NP and M genes, which are highly conserved among influenza A viruses [18].

Coronaviruses (*Coronaviridae*) are a group of viruses with the largest genomes among RNA viruses. Their genetic structure includes four main structural proteins: S, M, E, and N, which play a key role in disease pathogenesis and are the primary targets for diagnostics [19]. The S protein, responsible for virus binding to host cells, has received the most attention, making it a promising target for both diagnostics and therapeutic drug development [17].

Comparative analysis of the nucleotide sequences of viral genomes is conducted to design specific primers. Conserved regions of viral genomes become targets in the development of diagnostic molecular genetic tests [20]. The selection of specific primers and probes is a key step in the development of PCR-based diagnostic tests.

The aim of this study is to select primers and probes for real-time RT-PCR for the detection of influenza A and SARS-CoV-2 virus RNA.

Experimental

The SARS-CoV-2/KZ Almaty/04.2020 coronavirus strain and the A/Almaty/5/98(H1N1) influenza virus strain were used in this experiment.

Viral RNA was isolated under BSL-2 laboratory conditions using the innuPREP Virus DNA/RNA Kit according to the manufacturer's instructions. The quality and concentration of the obtained viral RNA were verified using a Nano Drop 2000 spectrophotometer.

Nucleotide sequences for selecting specific primers were searched in the NCBI GenBank international database (http://www.ncbi.nlm.nih.gov/GenBank).

Primers and probes were constructed using programs MEGA v.10, BLAST and Invitrogen Vector NTI for diagnostics of influenza A and SARS-CoV-2. The synthesis of oligonucleotide primers was carried out on an automatic synthesizer from K&A Laborgeraete, model DNA/RNA Synthesizer H-16 (made in Germany) using the phosphoramidite method according to the instructions supplied with the device.

Reverse transcription PCR and Real-Time Reverse transcription PCR

Reverse transcription PCR (RT-PCR) was performed using a ProFlex™ PCR System gradient thermal cycler, Applied Biosystems. Real-Time Reverse transcription PCR (Real-time RT-PCR) was performed on a Rotor-Gene Q thermal cycler, Qiagen.

RT-PCR amplification results were analyzed by electrophoresis at 400 mA in a 1.5 % agarose gel supplemented with SYBR Safe DNA gel stain, Invitrogen. The 100 bp DNA Ladder length marker, NEB, was used to estimate fragment length. Real-time RT-PCR results were detected and analyzed using Rotor-Gene Q software, version 1.8.187.5.

Results and Discussion

Numerous international studies are devoted to the development of highly specific and sensitive primers and probes for the detection of influenza A and SARS-CoV-2 viruses using real-time RT-PCR. Selecting a target gene is a key step in developing a diagnostic test, as it affects its analytical sensitivity and specificity.

For influenza A virus, the optimal target is the highly conserved matrix (M) gene, as confirmed by international studies, including the work of Spackman et al. (2002) and subsequent WHO protocols [21, 22]. For SARS-CoV-2, international recommendations indicate the RdRp gene as the most specific confirmatory target, and the E gene as a screening target, according to the protocol of Corman et al. (2020) [23].

In this study, the M gene for influenza A virus and the RdRp gene for SARS-CoV-2 were selected as target genes. This choice is consistent with the results of studies by Vogels et al. (2020), indicating high diagnostic sensitivity of the RdRp target [24]. Nucleotide sequences of the analyzed genes were retrieved from the GenBank database, taking into account the geographic and temporal diversity of the isolates. The list of GenBank IDs used is provided in Table 1.

Table 1

GenBank-registered influenza A virus and coronavirus isolates used for multiple nucleotide alignment

Name of the virus	**Isolate identification numbers in the GenBank database**
Influenza A virus (M gene)	EF541447.1, AJ410572.1, DQ021776.2, HM144894.1, AB299810.2, MG280269.1, JX673922.1, GU051365.1, KU679934.1, KJ781226.1, MG976713.1, KY644319.1, KT932366.1, KP414902.1, KC881293.1, GU182162.1, EF593105.1, DQ064385.1, MN530740.1, MK237090.1, KY130988.1, GU186693.1, GQ257419.1, GQ257456.1, AB916670.1, EU124190.1, DQ320993.1, MN253808.1, MG366527.1, MH932132.1, MH134599.1, KJ907546.1, KY644227.1, KX215215.1, KU289771.1, KP336340.1, KP336329.1, KP417011.1, GU083624.1, HM144813.1, CY178327.1, CY178031.1, CY178015.1, CY177927.1, CY177895.1
SARS-CoV-2	MT192765.1, MT159721.1 MT159714.1, MT159713.1 MT159710.1, MT039873.1, MN996528.1, MN988668.1, MT192772.1, MT184912.1, MT159709.1, MT159707.1, MT118835.1, MT019533.1, MT159722.1, MT159718.1, MT121215.1, MT123290.1, MT066175.1, MN996530.1, MT066156.1, MT093631.2, LC529905.1, MT159720.1, MT027062.1, MN996529.1, MT123291.2, MT135041.1, MN996531.1, MT184908.1, LC528232.1, LR757996.1, MT039887.1, MT123293.2, LR757995.1, MN988713.1, MT163719.1, MT019530.1, MT192759.1, MT106054.1, MT072688.1, MT184911.1, LR757998.1, MT050493.1, MT012098.1, MN996527.1, MN938384.1, MT188340.1, MT188339.1, MT044258.1.
SARS-CoV	AY395003.1, AY394996.1, AY304488.1, AY304486.1, AY394985.1, EU371559.1, AY394994.1, JX163927.1, JX163923.1, JQ316196.1, AY559096.1, AY274119.3, AY394999.1, AY394987.1, AY282752.2, JX163925.1, JX163924.1, EU371560.1, AY395002.1, AY357076.1, GU553365.1, AY338175.1, FJ429166.1, AY461660.1, AY568539.1, HQ890541.1, AY772062.1, FJ882953.1, FJ882962.1
Bat coronavirus	MN996532.1, MG772933.1, MG772934.1, KF367457.1, MK211378.1, MK211375.1, KY417151.1, JX993988.1, KJ473813., KJ473811.1, MK211376.1, KY417146.1, KY417145.1, KY417152.1

Multiple sequence alignment was performed using MEGA v.10. This analysis allowed us to identify conserved regions of the genome used in the design of primers and fluorescent probes (Figs 1-2).

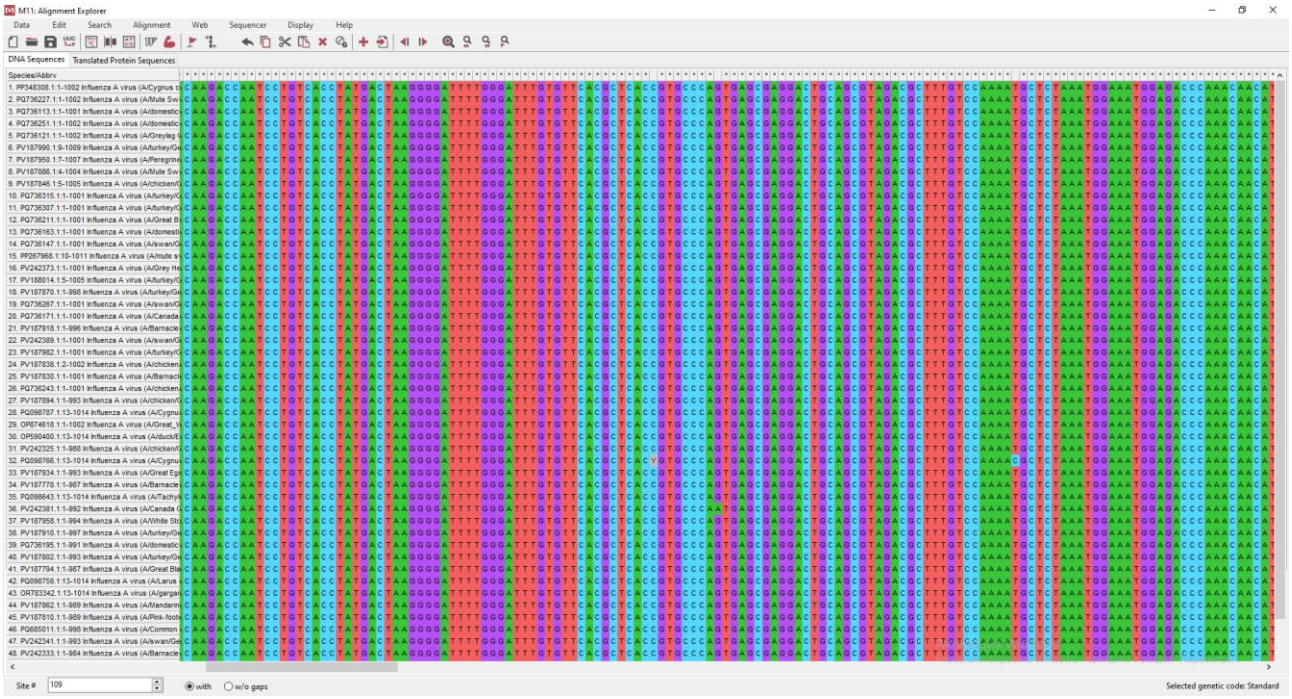


Figure 1. Results of a multiple alignment of the nucleotide sequences of the influenza A virus M gene, performed in MEGA v.10.

Color coding reflects nucleotide type:
adenine (A) — green, thymine (T) — red, guanine (G) — purple, cytosine (C) — blue

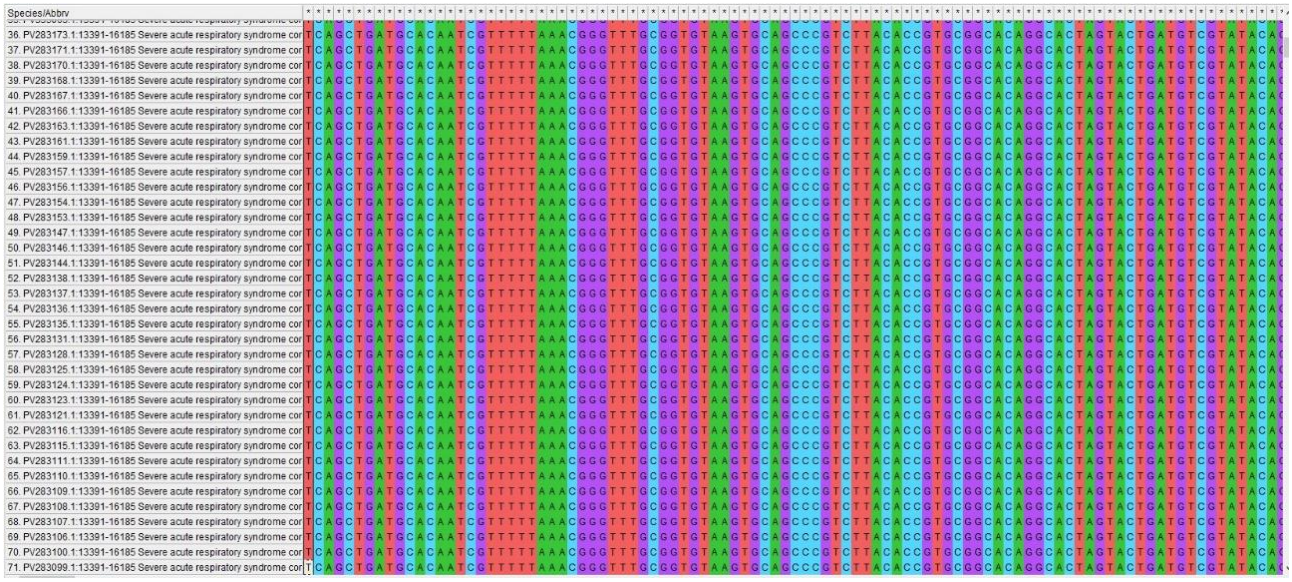
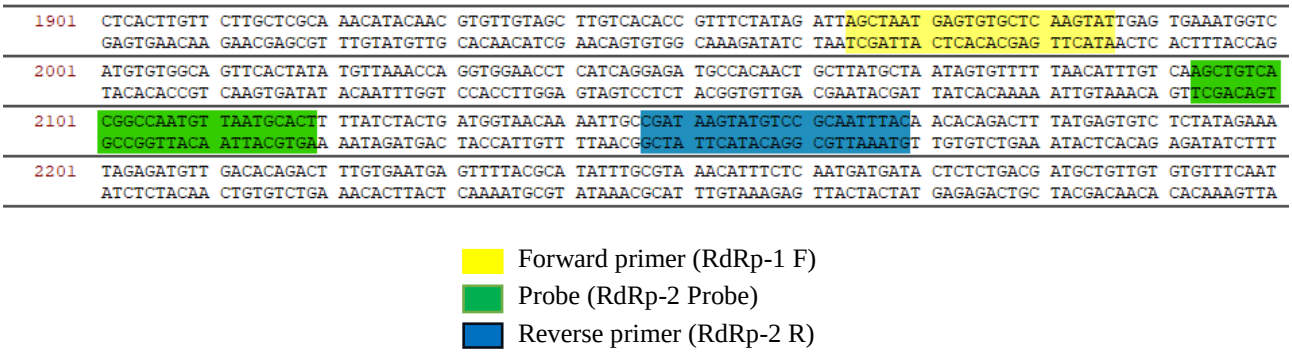


Figure 2. Results of a multiple alignment of the nucleotide sequences of the SARS-CoV-2 virus genomes, performed in MEGA v.10.

Color coding reflects nucleotide type:
adenine (A) — green, thymine (T) — red, guanine (G) — purple, cytosine (C) — blue

For each target, a primer pair and probe with the best specificity based on BLAST analysis were selected. For influenza A, primers InfM2 F/InfM2 R (153 bp amplicon) were used, and for SARS-CoV-2 RdRp-1 F/RdRp-2 R (205 bp amplicon) were used. Conserved regions selected for primer and probe attachment are shown in Figures 3-4. The parameters of the synthesized oligonucleotides are presented in Table 2.

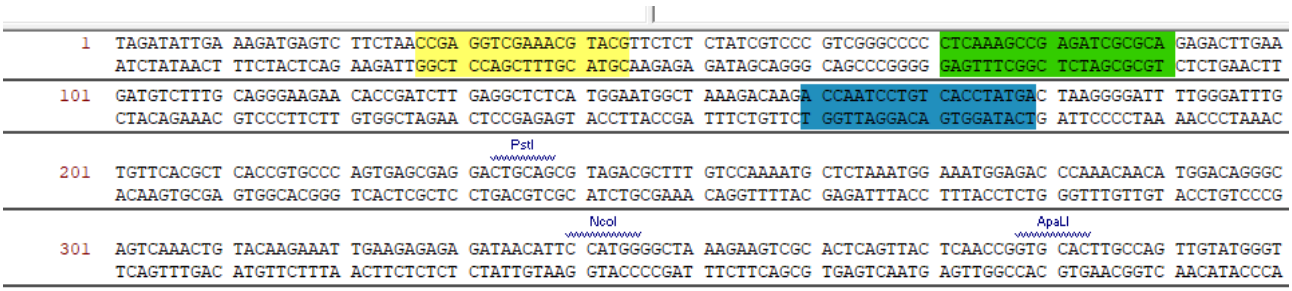


Forward primer (RdRp-1 F)
Probe (RdRp-2 Probe)
Reverse primer (RdRp-2 R)

Figure 3. Selection of sites for primers and a probe for the RdRp gene of the SARS-CoV-2

Figure 3 shows a fragment of the nucleotide sequence of the SARS-CoV-2 RdRp gene used to select oligonucleotide primers and a fluorescent probe for the detection of SARS-CoV-2 RNA by real-time RT-PCR.

The highlighted region was selected based on conservation analysis across different SARS-CoV-2 strains (GISAID and GenBank data), taking into account minimal variability and the absence of mutations at the primer and probe binding sites. The amplicon length is 205 base pairs, which meets the requirements for real-time RT-PCR. The probe is labeled with the ROX fluorophore and the BHQ-2 quencher.



Forward primer (InfM2 F)
Probe (InfM2 Probe)
Reverse primer (InfM2 R)

Figure 4. Selection of sites for primers and a probe for the M gene of the influenza A virus

Figure 4 shows a fragment of the nucleotide sequence of the M gene of the influenza A virus, one of the most conserved fragments of the genome, selected as the target for amplification.

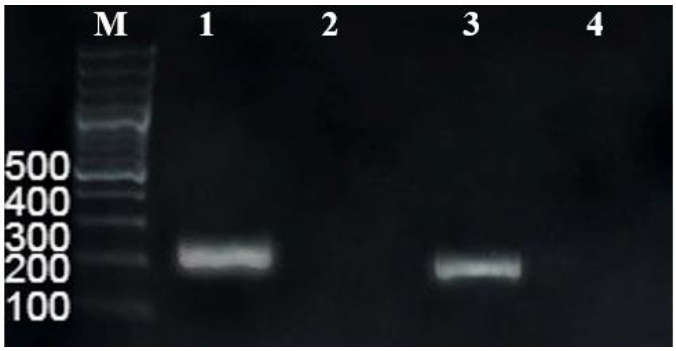
The designed primers and probes were tested for specificity using the BLAST program (https://blast.ncbi.nlm.nih.gov). Based on these studies, primers were designed; their parameters are listed in Table 2. FAM, ROX, and quenchers BHQ-1 and BHQ-2 were used as fluorescent dyes.

Table 2

Parameters of designed primers and probes

Name of primers and probe	Sequence 5' — 3'	Tm	GC, %	Size of product
InfM2 F	ACCGAGGTCGAAACGTAYGT	58	55	153 bp
InfM2 Probe	FAM- CTCAAAGCCGAGATMGCGCAG-BHQ-1	65	62	
InfM2 R	TCAGAGGTGACARGATTGGTC	59	52	
RdRp-1 F	AGCTAATGAGTGTGCTCAAGTAT	59	40	205 bp
RdRp-2 Probe	ROX-AGCTGTCACGGCCAATGTTAATGCACT-BHQ-2	68	48	
RdRp-2 R	GTAAATTGCGGACATACTTATCG	59	40	

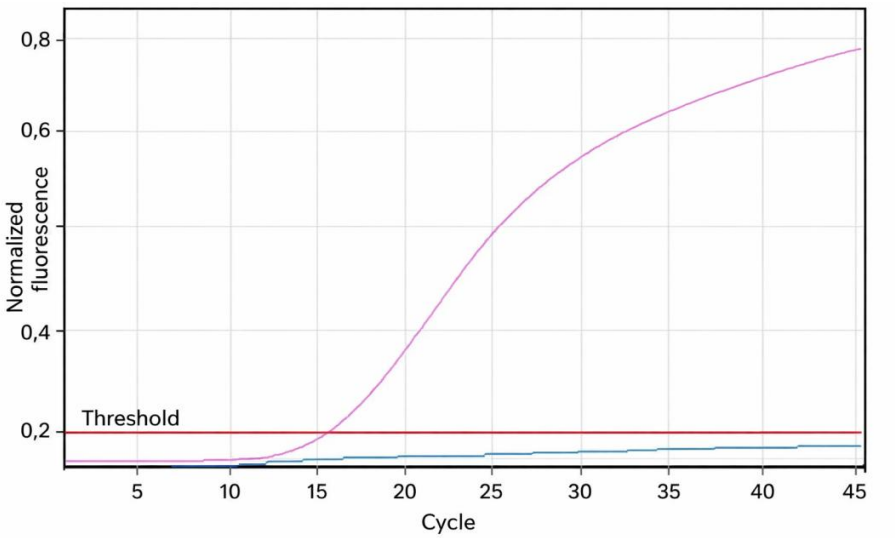
Based on the results of research, the performance of the primers and probes we developed for detecting influenza A virus and SARS-CoV-2 coronavirus RNA was tested using conventional PCR. The results are presented in Figure 5.



M — 100 bp DNA Ladder, NEB, 1 — positive control — plasmid DNA with an inserted fragment of the RdRp gene of the SARS-CoV-2 coronavirus, 2 — negative control, 3 — positive control–plasmid DNA with an inserted fragment of the M gene of the influenza A virus, 4 — negative control

Figure 5. Electropherogram of amplification products using primers RdRp-1 F and RdRp-2 R, InfM2 F and InfM2 R

The efficiency and specificity of the primers were further assessed using real-time PCR. Positive controls for influenza A and SARS-CoV-2 demonstrated early crossing of the fluorescence threshold (Ct = 15,75 and Ct = 18,70, respectively), while negative controls showed no signal (Figs 6-7, Tables 3-4). This confirms high specificity and the absence of nonspecific amplification.



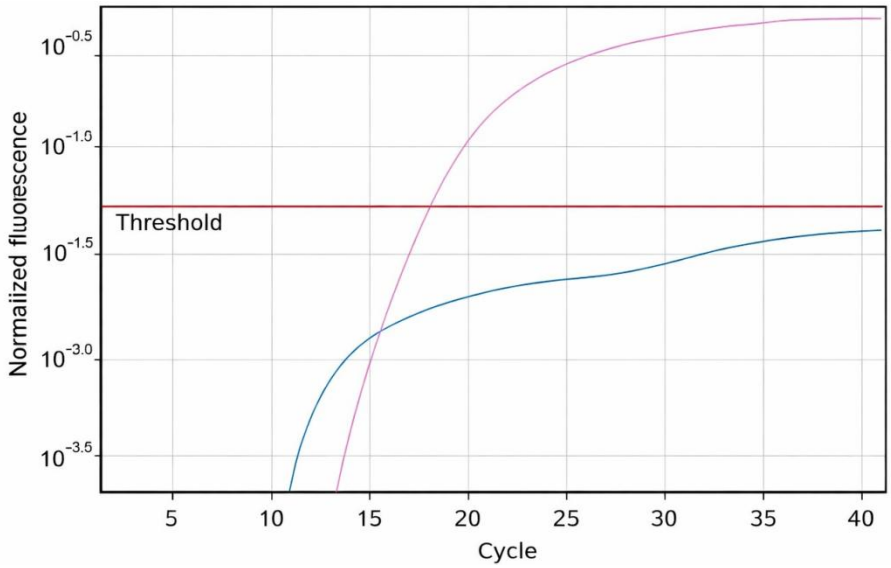
No. 1 — positive control — plasmid DNA with an inserted fragment of the M gene of the influenza a virus; No.2 –NC — negative control– deionized water

Figure 6. Results of real-time PCR analysis of primers and probe for detection of influenza A virus RNA

Table 3

Quantitative RT-PCR data in the Green channel

No.	Name	Type	CT	Avg. Ct
1	Influenza A	Positive control	15,75	15,75
2	NC	Negative control		



No. 1 — positive control — plasmid DNA with an inserted fragment of the RdRp gene of the SARS-CoV-2 coronavirus; No.2 — NC — negative control — deionized water

Figure 7. Results of real-time PCR analysis of primers and probe for detection of SARS-CoV-2 coronavirus RNA

Table 4

Quantitative RT-PCR data in the Orange channel

No.	Name	Type	CT	Avg. Ct
1	SARS-CoV-2	Positive control	18,70	18,70
2	NC	Negative control		

The RT-PCR results in Tables 2 and 3 show that the amplification curve for the influenza A virus positive control crossed the established fluorescence threshold as early as cycle 15,75 (Ct=15,75) during RT-PCR, and for the SARS-CoV-2 virus positive control, it crossed the established fluorescence threshold as early as cycle 18,70 (Ct=18,70), whereas no amplification occurred in the negative controls (no Ct) (Figs 6-7, Tables 3-4). The early appearance of a signal (low Ct) indicates a high initial target concentration and efficient target doubling in each PCR cycle. The absence of a signal in the negative control means that the reaction lacked the target nucleic acid and no non-specific amplification was observed; this confirms the high specificity of the primers and probes used. Thus, the experimental data demonstrate that the presence of positive controls of influenza A and SARS-CoV-2 viruses leads to an increase in fluorescence.

The presented data demonstrate the suitability of the selected primers and probes for further use in diagnostic PCR test-systems. High reaction efficiency (early Ct in the positive control) and the complete absence of amplification in the negative control indicate a reliable combination of efficacy and specificity of the selected oligonucleotides [25]. Thus, the developed primers and probes ensure selective and efficient detection of influenza A and SARS-CoV-2 viruses and can be recommended for integration into diagnostic test-systems using real-time PCR.

Conclusion

During the study, optimal primer pairs and fluorescent probes were selected for the detection of influenza A and SARS-CoV-2 viruses using real-time RT-PCR. Highly conserved regions were chosen as targets: the M gene of influenza A virus and the RdRp gene of SARS-CoV-2 virus, ensuring high sensitivity, specificity, and resistance to viral genome variability.

The developed oligonucleotide set provides the basis for the creation of reliable domestically produced diagnostic test-systems adapted to the epidemiological conditions of the Republic of Kazakhstan. Use of the-

se test-systems will improve the availability and efficiency of molecular diagnostics for acute respiratory infections, as well as strengthen epidemiological surveillance during the seasonal peak in cases.

Conflict of interest

The authors declare no conflict of interest.

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Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. **M.D. Almezhanova, A.M. Melisbek, M.Zh. Shirinbekov —** investigation, **N.S. Kozhabergenov, A.T. Junushov —** discussion of research results, **O.V. Chervyakova, K.T. Sultankulova —** conceptualization and management of work.

References

1 Припутневич Т. В. Опыт разработки и использования новой тест-системы для скрининга и диагностики инфекций, вызывающих острые респираторные заболевания / Т. В. Припутневич // Эпидемиология и Вакцинопрофилактика. — 2022. — Т. 21, № 3. — С. 72–79. https://doi.org/10.31631/2073-3046-2022-21-3-72-79

2 Rudan I. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries / I. Rudan, K. L. O'Brien, H. Nair // J Glob Health. — 2013. — Vol. 3, No. 1. — P. 10401.

3 Припутневич Т. В. Острые респираторные заболевания и грипп в современном акушерстве: эпидемиологические особенности и проблемы диагностики: обзор литературы / Т. В. Припутневич // Эпидемиология и Вакцинопрофилактика. — 2019. — Т. 18, № 3. — С. 89–97. https://doi.org/10.31631/2073-3046-2019-18-3-89-97

4 Seto D. S. Acute respiratory infections / D. S. Seto, R. M. Heller // Pediatr Clin North Am. — 1974. — Vol. 21, No. 3. — P. 683–709. doi: 10.1016/s0031-3955(16)33030-9. PMID: 4369753.

5 Орлова Н. В. Острые респираторные заболевания: особенности течения, медикаментозная терапия [Электронный ресурс] / В. Н. Орлова // Медицинский совет. — 2018. — № 15. — Режим доступа: https://cyberleninka.ru/article/n/ostrye-respiratornye-zabolevaniya-osobennosti-techeniya-medikamentoznaya-terapiya (Дата обращения: 08.11.2024).

6 Influenza (Seasonal). — 28 February 2025. Site World Health Organization. — [Electronic resource]. — Access mode: https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal).

7 Ayittey F.K. Economic impacts of Wuhan 2019-nCoV on China and the world / F. K. Ayittey, M. K. Ayittey, N. B. Chiwero, J. S. Kamasah, C. Dzuvor // Journal of medical virology. — 2020. — Vol. 92, No. 5. — P. 473. https://doi.org/10.1002/jmv.25706

8 Википедия: свободная энциклопедия «Пандемия COVID-19». — [Электронный ресурс]. — Режим доступа: https://ru.wikipedia.org/wiki/%D0 %9F%D0 %B0 %D0 %BD%D0 %B4 %D0 %B5 %D0 %BC%D0 %B8 %D1 %8F_COVID-19

9 Valencia D. N. Brief review on COVID-19: the 2020 pandemic caused by SARS-CoV-2 / D.N. Valencia // Cureus. — 2020. — Vol. 12, No. 3. — e7386. doi: 10.7759/cureus.7386.

10 Ni M. Simultaneous detection and differentiation of SARS-CoV-2, influenza A virus and influenza B virus by one-step quadruplex real-time RT-PCR in patients with clinical manifestations / M. Ni, H. Xu, J. Luo, W. Liu, D. Zhou // International Journal of Infectious Diseases. — 2021. — Vol. 103. — P. 517–524. doi: 10.1016/j.ijid.2020.12.027

11 Ma L. Development of a Novel Multiplex PCR Method for the Rapid Detection of SARS-CoV-2, Influenza A Virus, and Influenza B Virus / L. Ma, H. Zhu, Y. Jiang, X. Kong, P. Gao, Y. Liu, M. Zhao, G. Deng, Y. Cao // International Journal of Analytical Chemistry. — 2024. — No. 1. — 4950391. doi: 10.1155/2024/4950391

12 Bustin S. A. Real-time revers transcription PCR (qRT-PCR) and its potential use in clinical diagnosis / S. A Bustin, R. Mueller // Clinical Science. — 2005. — No. 4. — P. 365–379.

13 COVID-19: Серология, антитела и иммунитет. — [Электронный ресурс]. — Режим доступа: https://www.who.int/ru/news-room/questions-and-answers/item/coronavirus-disease-covid-19-serology (Дата обращения 08.11.2024)

14 Klumpp-Thomas C. Standardization of ELISA protocols for serosurveys of the SARS-CoV-2 pandemic using clinical and at-home blood sampling / C. Klumpp-Thomas, H. Kalish, M. Drew, S. Hunsberger, K. Snead, M. P. Fay // Nature communications. — 2021. — 12. — P. 1–13.

15 Sit T. H. C. et al. Infection of dogs with SARS-CoV-2 / T.H.C. Sit et al. // Nature. — 2020. — Vol. 586, No. 7831. — P. 776–778. doi: 10.1038/s41586-020-2334-5.

16 Gavin P. J. Review of Rapid Diagnostic Tests for Influenza / P. J. Gavin, R. B. Thomson // Clinical and Applied Immunology Reviews. — 2004. — 4(3). — P. 151–172. https://doi.org/10.1016/S1529-1049(03)00064-3.

17 Quinlivan M. Comparison of Sensivities of Virus Isolation, Antigen Detection, and Nucleic Acid Amplification for Detection of Equine Influenza Virus / M. Quinlivan, A. Cullinane, M. Nelly // Journal of Clinical Microbiology. — 2004. — Vol. 42. — P. 759–763. doi: 10.1128/JCM.42.2.759-763.2004.

18 Hoffmann E. et al. Universal primer set for the full-length amplification of all influenza A viruses / E. Hoffmann et al. // Archives of Virology. — 2001. — Vol. 146(12). — P. 2275–2289. doi:10.1007/s007050170002

19 Stone B. et al. Rapid detection and simultaneous subtype differentiation of influenza A viruses by real time PCR / B. Stone et al. // Journal of virological methods. — 2004. — Vol. 117(2). — P. 103–112. doi:10.1016/j.jviromet.2003.12.005

20 Yanagita M. et al. Development of a Real-Time Reverse Transcription PCR Assay System for Detection of Three Subtypes of Influenza A and Influenza B Viruses / M. Yanagita et al. // Rinsho byori. The Japanese Journal of Clinical Pathology. — 2015. — Vol. 63(12). — P. 1365–1370. Japanese. PMID: 27089652.

21 Spackman E. et al. Development of a Real-Time Reverse Transcriptase PCR Assay for Type A Influenza Virus and the Avian H5 and H7 Hemagglutinin Subtypes / E. Spackman et al. // Journal of Clinical Microbiology. — 2002. — Vol. 40(9). — P. 3256–3260. https://doi.org/10.1128/JCM.40.9.3256-3260.2002

22 World Health Organization. CDC protocol of real-time RT-PCR for influenza A(H1N1). — 2009. — [Electronic resource]. — Access mode: https://www.ecphf.cn/upload/RealtimeRTPCR_SwineH1Assay-2009_20090430.pdf?utm_source

23 Corman V. M. et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR / V. M. Corman et al. // Eurosurveillance. — 2020. — Vol. 25(3). — P. 2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

24 Vogels C.B.F. Analytical sensitivity and efficiency comparisons of SARS-CoV-2 qRT-PCR primer-probe sets / C. B. F. Vogels // Nature Microbiology. — 2020. — Vol. 5. — P. 1299–1305. https://doi.org/10.1038/s41564-020-0761-6

25 Dieffenbach C. W. et al. General concepts for PCR primer design / C. W. Dieffenbach et al. // PCR Methods Appl. — 1993. — Vol. 3(3). — P. 30–37. doi: 10.1101/gr.3.3.s30. PMID: 8118394.

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Нақты уақыттағы КТ-ПТР әдісі арқылы А тұмауы мен SARS-CoV-2 вирустарының РНҚ-ын анықтауға арналған праймерлер мен зондтарды таңдау

Клиникалық вирусология зертханаларында кең таралған және жаңа вирустарды сынау және диагностикалау үшін үнемді, нақты және жылдам талдау қажет. КТ-ПТР талдауын қолдана отырып, А типті тұмауы вирусын және SARS-CoV-2 коронавирусын анықтау үшін олигонуклеотидтер таңдалған және құрастырылған. А тұмауы вирусы мен SARS-CoV-2 диагностикасы үшін отандық сынақ-жүйелерін құру маңызды міндет және ауруларды ерте диагностикалау қажеттілігіне байланысты. Зерттеудің мақсаты нақты уақыттағы КТ-ПТР (НУ КТ-ПТР) көмегімен А тұмауы және SARS-CoV-2 диагностикасына арналған праймерлер мен зондтарды таңдау. Мақалада А тұмауы және SARS-CoV-2 вирустарының РНҚ сәйкестендіруге арналған НУ КТ-ПТР үшін праймерлер мен зондтарды жобалау және синтездеу нәтижелері ұсынылған. Арнайы праймерлер мен зондтарды таңдау бойынша зерттеулерімізде А тұмауы вирусын анықтау үшін мақсатты ген ретінде М гені және SARS-CoV-2 вирусы үшін RdRp гені таңдалды. А тұмауы үшін InfM2 F және InfM2 R праймерлері мен InfM2 зонды, ал SARS-CoV-2 үшін RdRp-1 F және RdRp-2 R праймерлері мен RdRp-2 зонды жұпты олигонуклеотидтері таңдалып синтезделді. Таңдалған жұптық олигонуклеотидтер НУ КТ-ПТР көмегімен А тұмауы вирусын және SARS-CoV-2 анықтау кезінде 20 пмоль жұмыс концентрациясында жоғары тиімділігін көрсетті. Праймерлерді таңдау Primer Blast және Vector NTI компьютерлік бағдарламаларын қолдану арқылы жүзеге асырылды. Құрастырылған праймерлер мен зондтар отандық мультиплексті НУ КТ-ПТР сынақ-жүйесін құру кезінде қолданылады.

Кілт сөздер: А тұмауы, коронавирус, КТ-ПТР, диагностика, сынақ-жүйе

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Подбор праймеров и зондов для выявления РНК вирусов гриппа А и SARS-CoV-2 методом ОТ-ПЦР в реальном времени

Для тестирования и диагностики обычных и новых вирусов в клинических вирусологических лабораториях требуется экономичный, точный и быстрый анализ. Нами подобраны и сконструированы олигонуклеотиды для выявления вируса гриппа типа А и коронавируса SARS-CoV-2 с применением ОТ-ПЦР-анализа. Создание отечественных тест-систем для диагностики вируса гриппа А и SARS-CoV-2

является острой задачей и обусловлено необходимостью ранней диагностики заболеваний. Целью данного исследования является подбор праймеров и зондов для диагностики гриппа А и SARS-CoV-2 методом ОТ-ПЦР в реальном времени (ОТ-ПЦР РВ). В работе представлены результаты конструирования праймеров и зондов для постановки ОТ-ПЦР РВ для идентификации РНК вирусов гриппа А и SARS-CoV-2. В наших исследованиях по подбору специфических праймеров и зондов в качестве гена-мишени для обнаружения вируса гриппа А был выбран М-ген, вируса SARS-CoV-2 — RdRp. Были подобраны и синтезированы пары олигонуклеотидных праймеров для гриппа А: InfM2 F и InfM2 R, а также зонд InfM2 Probe, а для SARS-CoV-2 — RdRp-1 F и RdRp-2 R, зонд RdRp-2 Probe, которые при постановке ОТ-ПЦР РВ с рабочей концентрацией 20 пмоль показали высокую эффективность при выявлении вируса гриппа А и SARS-CoV-2. Подбор праймеров проведен с использованием компьютерных программ PrimerBlast и VectorNTI. Конструированные праймеры и зонды в дальнейшем будут использованы при создании отечественной мультиплексной ОТ-ПЦР РВ тест-системы.

Ключевые слова: грипп А, коронавирус, ОТ-ПЦР, диагностика, тест-система

References

1 Priputnevich, T. V. (2022) Opyt razrabotki i ispolzovaniia novoi test-sistemy dlia skrininga i diagnostiki infektsii, vyzyvaiushchikh ostrye respiratornye zabolevaniia [Experience in the development and use of a new test system for screening and diagnosing infections causing acute respiratory diseases]. *Epidemiologiia i Vaktsinoprofilaktika — Epidemiology and Vaccine Prevention, 21*(3), 72–79. https://doi.org/10.31631/2073-3046-2022-21-3-72-79 [in Russian].

2 Rudan, I., O'Brien, K. L., & Nair, H. (2013). Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. *J Glob Health, 3*(1), 10401.

3 Priputnevich, T. V. (2019). Ostrye respiratornye zabolevaniia i gripp v sovremennom akusherstve: epidemiologicheskie osobennosti i problemy diagnostiki: obzor literatury [Acute respiratory diseases and influenza in modern obstetrics: epidemiological features and diagnostic problems: a literature review]. *Epidemiologiia i Vaktsinoprofilaktika — Epidemiology and Vaccine Prevention, 18*(3), 89–97. https://doi.org/10.31631/2073-3046-2019-18-3-89-97 [in Russian].

4 Seto, D. S., & Heller, R. M. (1974). Acute respiratory infections. *Pediatr Clin North Am, 21*(3), 683–709. doi: 10.1016/s0031-3955(16)33030-9. PMID: 4369753.

5 Orlova, N. V. (2018). Ostrye respiratornye zabolevaniia: osobennosti techeniia, medikamentoznaia terapiia [Acute respiratory diseases: features of the course, drug therapy]. *Meditsinskii sovet — Medical Council,* 15. Retrieved from https://cyberleninka.ru/article/n/ostrye-respiratornye-zabolevaniya-osobennosti-techeniya-medikamentoznaya-terapiya (Date of request: 08.11.2024) [in Russian].

6 Influenza (Seasonal). 28 February 2025. Site World Health Organization. *who.int.* Retrieved from https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal).

7 Ayittey, F. K., Ayittey, M. K., Chiwero, N. B., Kamasah, J. S., & Dzuvor, C. (2020). Economic impacts of Wuhan 2019-nCoV on China and the world. *Journal of medical virology, 92,* 5, 473. Retrieved from https://doi.org/10.1002/jmv.25706

8 Vikipediia: svobodnaia entsiklopediia «Pandemiia COVID-19» [Wikipedia: The Free Encyclopedia "COVID-19 Pandemic"]. Retrieved from https://ru.wikipedia.org/wiki/%D0 %9F%D0 %B0 %D0 %BD%D0 %B4 %D0 %B5 %D0 %BC%D0 %B8 %D1 %8F_ COVID-19 [in Russian].

9 Valencia, D. N. (2020). Brief review on COVID-19: the 2020 pandemic caused by SARS-CoV-2. *Cureus, 12*(3), e7386. doi: 10.7759/cureus.7386.

10 Ni, M., Xu, H., Luo, J., Liu, W., & Zhou, D. (2021). Simultaneous detection and differentiation of SARS-CoV-2, influenza A virus and influenza B virus by one-step quadruplex real-time RT-PCR in patients with clinical manifestations. *International Journal of Infectious Diseases, 103*, 517–524. doi: 10.1016/j.ijid.2020.12.027

11 Ma, L., Zhu, H., Jiang, Y., Kong, X., Gao, P., Liu, Y., Zhao, M., Deng, G., & Cao, Y. (2024). Development of a Novel Multiplex PCR Method for the Rapid Detection of SARS-CoV-2, Influenza A Virus, and Influenza B Virus. *International Journal of Analytical Chemistry*, 1, 4950391. doi: 10.1155/2024/4950391

12 Bustin, S. A., & Mueller, R. (2005). Real-time revers transcription PCR (qRT-PCR) and its potential use in clinical diagnosis. *Clinical Science*, 4, 365–379.

13 COVID-19: Serologiia, antitela i immunitet [Serology, antibody and immunity]. Retrieved from https://www.who.int/ru/news-room/questions-and-answers/item/coronavirus-disease-covid-19-serology (Date of access 08.11.2024) [in Russian].

14 Klumpp-Thomas, C., Kalish, H., Drew, M., Hunsberger, S., Snead, K., & Fay, M. P. (2021). Standardization of ELISA protocols for serosurveys of the SARS-CoV-2 pandemic using clinical and at-home blood sampling. *Nature communications, 12*, 1–13.

15 Sit, T. H. C., Brackman, C. J., Ip, S. M., Tam, K. W. S., Law, P. Y. T., & To, E. M. W. (2020). Infection of dogs with SARS-CoV-2. *Nature, 586* (7831), 776–778. doi: 10.1038/s41586-020-2334-5.

16 Gavin, P. J., & Thomson, R. B. (2004). Review of Rapid Diagnostic Tests for Influenza. *Clinical and Applied Immunology Reviews, 4*(3), 151–172. https://doi.org/10.1016/S1529-1049(03)00064-3.

17 Quinlivan, M., Cullinane, A., & Nelly, M. (2004). Comparison of Sensivities of Virus Isolation, Antigen Detection, and Nucleic Acid Amplification for Detection of Equine Influenza Virus. *Journal of Clinical Microbiology, 42*, 759–763. doi: 10.1128/JCM.42.2.759-763.2004.

18 Hoffmann, E., Stech, J., Guan, Y., Webster, R. G., & Perez, D. R. (2001). Universal primer set for the full-length amplification of all influenza A viruses. *Archives of Virology, 146*(12), 2275–2289. doi:10.1007/s007050170002

19 Stone, B., Burrows, J., Schepetiuk, S., Higgins, G., Hampson, A., Shaw, R., & Kok, T. (2004). Rapid detection and simultaneous subtype differentiation of influenza A viruses by real time PCR. *Journal of virological methods, 117*(2), 103–112. doi:10.1016/j.jviromet.2003.12.005

20 Yanagita, M., Kuwamura, Y., Kinoshita, S., Nakajima, T., Tomizawa, S., & Ozawa, T. (2015). Development of a Real-Time Reverse Transcription PCR Assay System for Detection of Three Subtypes of Influenza A and Influenza B Viruses. *Rinsho byori. The Japanese Journal of Clinical Pathology, 63*(12), 1365–1370. Japanese. PMID: 27089652.

21 Spackman, E., Senne, D. A., & Myers, T. J. (2002). Development of a Real-Time Reverse Transcriptase PCR Assay for Type A Influenza Virus and the Avian H5 and H7 Hemagglutinin Subtypes. *Journal of Clinical Microbiology*, *40*(9), 3256–3260. https://doi.org/10.1128/JCM.40.9.3256-3260.2002

22 (2009). World Health Organization CDC protocol of real-time RT-PCR for influenza A(H1N1). https://www.ecphf.cn/upload/RealtimeRTPCR_SwineH1Assay-2009_20090430.pdf?utm_source

23 Corman, V. M., Landt, O., & Kaiser, M. (2020). Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance*, *25*(3), 2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

24 Vogels, C. B. F. (2020). Analytical sensitivity and efficiency comparisons of SARS-CoV-2 qRT-PCR primer-probe sets. *Nature Microbiology*, *5*, 1299–1305. https://doi.org/10.1038/s41564-020-0761-6

25 Dieffenbach, C. W., Lowe, T. M., & Dveksler, G. S. (1993). General concepts for PCR primer design. *PCR Methods Appl, 3*(3), 30–37. doi: 10.1101/gr.3.3.s30. PMID: 8118394.

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Cytoprotective and immunostimulatory properties of *Stachys sieboldii* and *Stevia rebaudiana* under metabolic stress in rats

This study evaluated the protective effects of *Stachys sieboldii* and *Stevia rebaudiana* extracts on bone marrow and spleen cells in rats subjected to a high-fat, high-sucrose (HFHS) diet. Prepubertal Wistar rats were randomly assigned to four groups (control, HFHS, HFHS+*Stachys*, HFHS+*Stevia*) and maintained for 30 days. Spleen and bone-marrow cellularity and cell viability were quantified. The HFHS diet increased the proportion of non-viable cells and reduced nucleated-cell counts in both organs. *Stachys* primarily enhanced splenic immune-cell proliferation (higher nucleated-cell concentration), whereas *Stevia* produced a stronger cytoprotective response, reducing the fraction of dead cells in bone marrow. Taken together, these findings suggest complementary actions: *Stachys* acts as a pro-proliferative modulator of splenic immune cells, while *Stevia* protects hematopoietic cells from HFHS-induced damage. These results highlight the potential of these plant extracts as dietary components for supporting immune and hematopoietic function and provide a basis for their further investigation in preventive and immunomodulatory applications.

Keywords: immunity, bone marrow, spleen, high-fat diet, HFHS, *Stevia rebaudiana*, *Stachys sieboldii.*

Introduction

In recent decades, dietary patterns worldwide have undergone significant changes due to globalization, industrialization, and urbanization [1]. Traditional diets, rich in natural foods, are gradually being replaced by high-fat, high-carbohydrate diets, which have become readily accessible due to the expansion of the food industry [2].

Modern diets in both developed and developing countries differ significantly, yet share a common feature: increased consumption of refined carbohydrates and saturated fats. In Western countries, such as the United States, Canada, and much of Europe, diets are characterized by high intake of processed foods, sugary beverages, fast food, and sweets [3; 4; 5]. In developing nations, including China, India, and countries in Africa, traditional diets rich in vegetables, legumes, and whole grains are also giving way to more modern "Western" diets. This shift is linked to urbanization and globalization, which make Western foods more available and appealing [6; 7]. Despite differences in traditional diets, the outcomes of this dietary shift are similar: sharp increases in obesity, cardiovascular disease, and metabolic disorders are observed in both developed and developing countries [8; 9].

A high-fat, high-carbohydrate diet not only disrupts metabolic processes but also significantly impacts the immune and hematopoietic systems, making this issue relevant for research and prevention [10; 11]. Biologically active supplements (BAS) can notably influence the spleen and bone marrow, especially through their antioxidant and anti-inflammatory properties, which may support normal immune and hematopoietic functions. For instance, quercetin, a natural flavonoid with powerful antioxidant and anti-inflammatory properties [12], has been shown in animal studies to increase lymphocyte and macrophage counts in the spleen [13]. In one study on rats undergoing chemotherapy, quercetin supplementation helped restore spleen function by increasing the concentration of nucleated cells, such as lymphocytes, by reducing oxidative stress and inflammation in spleen tissue [14].

Echinacea, widely used as an immune-stimulating agent, has shown beneficial effects on hematopoiesis in bone marrow [15]. Studies on mice revealed that echinacea intake increases the number of nucleated cells, such as macrophages and neutrophils, in the bone marrow [16]. This effect is associated with activation of stem cell proliferation and accelerated tissue repair processes after exposure to inflammatory agents or infec-

tions. An increase in the total cellular mass of the spleen and bone marrow was also observed, indicating a recovery of immune function [17].

Resveratrol, known for its antioxidant properties, positively affects bone marrow hematopoiesis, particularly under conditions of chronic inflammation [18]. In a study on mice with induced inflammation, resveratrol supplementation helped reduce bone marrow cell damage, maintaining normal stem cell proliferation [19]. In the spleen, an increase in lymphocyte counts and improved function were observed, attributed to resveratrol's immunomodulatory effects [20]. This highlights resveratrol's role in supporting normal hematopoietic processes under stress or inflammation.

Currently, research continues into the properties and medicinal potential of well-known BAS, such as *Stevia rebaudiana* and *Stachys sieboldii*.

Stachys (*Stachys sieboldii*) is a plant used in traditional medicine due to its rich content of bioactive compounds, such as phenolic compounds, flavonoids, and essential oils [21]. These substances have antioxidant, anti-inflammatory, and immunostimulatory properties. *Stachys* extracts are used for treating inflammation, enhancing immune function, and accelerating wound healing [22]. *Stachys* tubers, which have a nutty flavor, are rich in nutrients and are consumed as a dietary product, particularly beneficial for individuals with diabetes due to their blood sugar-regulating properties [21]. In modern medicine, *Stachys* is being investigated as an agent for enhancing immune response and reducing inflammation.

Stevia (*Stevia rebaudiana*) is well-known for its natural sweeteners, stevioside and rebaudioside, which are calorie-free and highly sweet-tasting [23]. Stevia also contains flavonoids and antioxidants that have anti-inflammatory and antioxidant effects. Stevia extracts are used to reduce blood sugar levels, making it popular among individuals with diabetes and those who monitor their weight. In cooking, stevia leaves are used as a natural sugar substitute in beverages, baked goods, and other dishes [24]. The medicinal properties of stevia include blood glucose regulation, improvement of the body's antioxidant status, and reduction of cardiovascular disease risk [25].

The aim of this study is to evaluate the effects of adding *Stachys sieboldii* and *Stevia rebaudiana* extracts to a high-fat, high-sucrose diet on the cellularity and viability of spleen and bone marrow cells in rats, as well as to determine their potential as cytoprotective and immunostimulatory agents.

The novelty of this work lies in the fact that, for the first time, a study is conducted on how adding plant-based products derived from *Stachys sieboldii* and *Stevia rebaudiana* to a high-fat, high-sugar diet affects the cellularity and integrity of spleen and bone marrow cells in rats under metabolic stress. Additionally, a direct comparative analysis of the cytoprotective and immunostimulatory properties of *Stachys sieboldii* and *Stevia rebaudiana* is carried out—an approach not previously presented in the scientific literature.

Experimental

Material preparation. In this experiment, a choice was made between two cultivated varieties of *Stachys sieboldii*: "Bochonok" and "Rakushka", both of which are successfully grown, including in Central Kazakhstan (2023–2025), due to their good adaptation to local conditions and status as garden crops. However, the "Rakushka" variety was selected for this study due to its specific biochemical composition. It contains a higher concentration of antioxidants, including phenolic compounds and flavonoids, as well as a significant amount of ascorbic acid and bioactive glycosides [26].

For the purpose of this study, the "Rakushka" variety was cultivated in experimental introduction fields at the Phytochemistry Holding research facility in Karaganda (Kazakhstan). After reaching maturity, the tubers were harvested and processed at the same facility. The roots were thoroughly washed three times with tap water to remove any adhering sand and dust. They were then lyophilized for 72 hours to preserve their bioactive compounds and ground into a fine powder. The resulting *Stachys sieboldii* root powder was stored at -70 °C until it was incorporated into the experimental diets for rodents.

The powdered organic extract of *Stevia rebaudiana* leaves was purchased online (SweetLeaf, Gilbert, Arizona, USA). The dosage was calculated based on the allowable daily dose recommended by the United States Food and Drug Administration (FDA) (5 mg/kg) [27]. These doses were determined as follows: the allowable daily dose was multiplied by the average weight of the rats and then divided by the group's average daily fluid intake. Dosages were recalculated weekly to account for weight gain and changes in fluid intake.

Animal experiments and diets. The experimental subjects consisted of 40 juvenile Wistar rats. The average body weight of the animals at the beginning of the experiment was 50–70 g. Juvenile rats were selected for the experiment at 21 days of age. The animals were housed in the vivarium of Karaganda Medical Uni-

versity under controlled conditions at 18±2 °C, 55 % ± 5 % humidity, and a 12-hour light-dark cycle (8:00–20:00). Throughout the experimental period, the animals had free access to food and water. The rats were randomly assigned to one of four experimental groups (n = 10 per group):

- Group 1: 10 juvenile males on a standard, balanced vivarium diet.
- Group 2: 10 juvenile males on a daily high-fat, high-sucrose diet (HFHS).
- Group 3: 10 juvenile males on a daily high-fat, high-sucrose diet supplemented with *Stachys sieboldii* (HFHS + Stachys).
- Group 4: 10 juvenile males on a daily high-fat, high-sucrose diet supplemented with *Stevia rebaudiana* (HFHS + Stevia) (Table 1).

Table 1

Composition of the experimental diet

Components (g/kg)	**1 group Intact**	**2 group HFHS**	**3 group HFHS + Stachys**	**4 group HFHS + Stevia**
Corn	200	80	80	80
Rice	200	200	200	200
Bone meal	120	120	120	120
Sucrose	—	100	100	100
Soy oil	75	—	—	—
Lard	—	200	200	200
Gluten	200	200	200	200
Salt	3.5	3.5	3.5	3.5
Mineral mix	35	35	35	35
Vitamin mix	16.5	16.5	16.5	16.5
Inert material	150	45	45	45
Total (g)	1000	1000	1000	1000
Nutrient composition (%)				
Protein	24.8	19.2	19.2	19.2
Carbohydrate	49.6	43.4	43.4	43.4
Lipids	25.6	37.4	37.4	37.4
Stachys sieboldii root powder	—	—	5	—
Stevia rebaudiana leaf powder	—	—	—	5
Energy density (kcal/g)	3.55	4.49	4.49	4.49

In this diet, the total carbohydrate content in the HFHS groups appears lower compared to the control group. This is explained by the substantial increase in dietary fat (lard) in the HFHS diet, which altered the overall macronutrient distribution while maintaining a total feed mass of 1000 g/kg. Additionally, this is due to the replacement of corn, which was reduced from 200 g/kg in the control group to 80 g/kg in the HFHS groups (corn contains both digestible carbohydrates and dietary fiber), with sucrose, which is fully digestible. When adjusted for fiber content, the actual intake of digestible carbohydrates is higher in the HFHS groups. Since sucrose is completely digestible and does not contribute to dietary fiber, the shift from complex carbohydrates to simple sugars resulted in a decrease in total carbohydrate mass despite the increased sugar content.

The experiment lasted for 30 days. The study was conducted in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg 1986), OECD GLP guidelines, EAEU Good Laboratory Practice Regulations No. 81, and the Order of the Minister of Health of the Republic of Kazakhstan No. MoH RK-151/2020, dated Octo-

ber 23, 2020, titled "On Approval of the Regulation on the Central Commission on Bioethics". The study was approved by the decision of the Bioethics Committee of "Karaganda Medical University" on 17.06.2021, protocol No.165.

Determination of cellularity and viability of bone marrow and spleen. To assess the cellularity of bone marrow and spleen, the spleen and femur were extracted from each animal, washed in physiological saline, and blotted dry on filter paper. Using a homogenizer, cell suspensions from each organ of individual rats were prepared in Hank's solution. The spleen and bone marrow suspensions were filtered through a nylon mesh to remove stromal elements, and the concentration of nucleated cells (NC) was counted using a standard method with a Goryaev chamber.

To identify non-viable cells, a 0.4 % trypan blue (TB) solution was used as a stain. The TB solution was prepared according to the manufacturer's instructions for the cell counter: a weighed portion of dry TB ($C_{34}H_{28}N_6O_{14}S_4$; Mikroskopie, Germany) was added to a solution of 0.81 % sodium chloride (NaCl; Sigma-Aldrich, USA) and 0.06 % potassium phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$; Merck, Germany) in distilled water, mixed until dissolved at room temperature, filtered through a 0.22 μm filter, and stored in a dark glass container at 4 °C. For viable cell counts, the cell suspension was mixed with TB solution at a 10:1 ratio, and a drop of the suspension was placed in the chamber. Using a microscope, stained and unstained cells were counted. The cell concentration in 1 ml (C) was determined using the formula: $C = kn \times 10^4$, where *n* is the number of cells counted in the Goryaev chamber, and *k* is the dilution factor (for staining with 0.4 % TB at a 10:1 ratio, $k = 1.1$).

Viability (V) of the cell population was calculated using the formula and expressed as a percentage: $V = (1–m/N) \times 100$, where *m* is the number of stained cells, and *N* is the total number of cells [28].

Statistical methods. One-way ANOVA was used for statistical data analysis to identify differences between groups. Tukey's multiple comparison test was applied for pairwise comparisons. Results were considered statistically significant at $p < 0.05$. All analyses were performed using GraphPad Prism 8 software.

Results

*Concentration of nucleated cells in the spleen of immature rats***.** The data obtained showed that the HFHS diet led to a 13.49 % increase in the concentration of nucleated cells in the spleen ($p < 0.0074$) compared to the control group. This may indicate a response to metabolic stress and inflammation (Fig. 1).

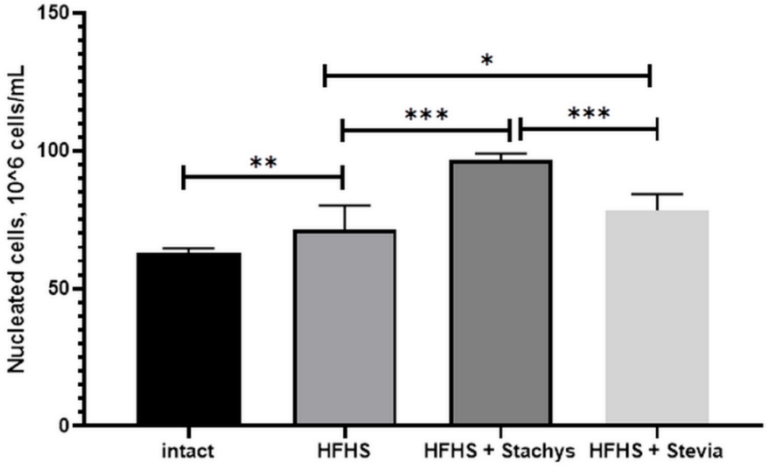

Figure 1. Concentration of nucleated cells in the spleen of immature rats in the experiment

The addition of *Stachys* to the HFHS diet resulted in a 35.55 % increase ($p < 0.0001$) in the number of nucleated cells in the spleen compared to the HFHS group, indicating a significant stimulation of immune cell proliferation. *Stevia* increased spleen nucleated cell concentration by 10.06 % ($p < 0.0289$) compared to the HFHS group, which also indicates a positive effect, though less pronounced than that of *Stachys* (Fig. 1).

*Cell viability in the spleen of immature rats***.** The HFHS diet led to an 89.23 % increase in the percentage of dead cells compared to the control ($p < 0.0001$), indicating a negative impact on spleen immune cells (Fig. 2).

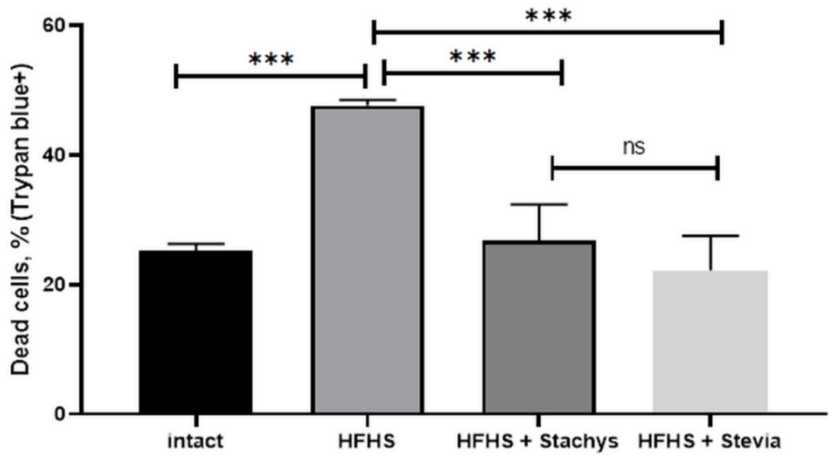

Figure 2. Cell viability indicators in the spleen of immature rats in the experiment

The addition of *Stachys* to the diet reduced the percentage of dead cells by 43.90 % ($p < 0.0001$) compared to the HFHS group, thus improving cell viability. *Stevia* showed an even more pronounced effect, reducing the percentage of dead cells by 53.45 % ($p < 0.0001$) relative to HFHS, indicating a stronger cytoprotective effect (Fig. 2).

Concentration of nucleated cells in the bone marrow of immature rats. In examining the concentration of nucleated cells in the bone marrow, it was found that the HFHS diet reduced the number of nucleated cells in the bone marrow by 34.38 % ($p < 0.0001$) compared to the control, indicating suppression of hematopoiesis (Fig. 3).

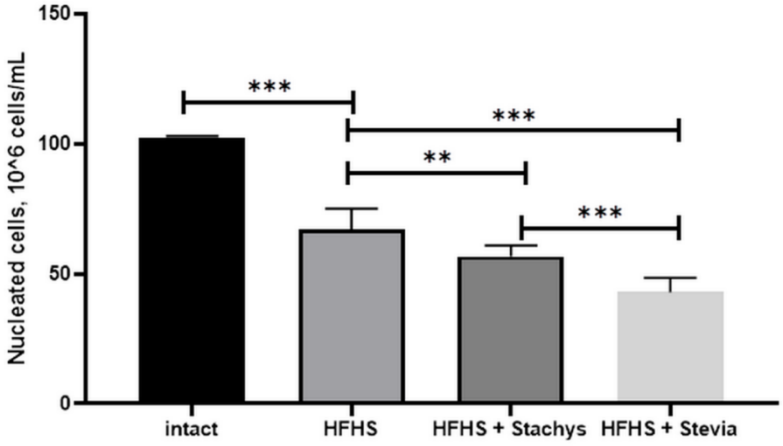

Figure 3. Concentration of nucleated cells in the bone marrow of immature rats in the experiment

Stachys, when added to the diet, further reduced the nucleated cells in the bone marrow by 15.55 % ($p < 0.0004$) compared to the HFHS group, which may indicate a negative impact on bone marrow. *Stevia* also decreased the number of nucleated cells in the bone marrow by 36.13 % ($p < 0.0001$) relative to HFHS, indicating a more significant suppression than observed with *Stachys* (Fig. 3).

Bone marrow cell viability in immature rats. The HFHS diet increased the percentage of dead cells by 4.6 times ($p < 0.0001$) compared to the control, showing a strong negative effect on bone marrow cells (Fig. 4).

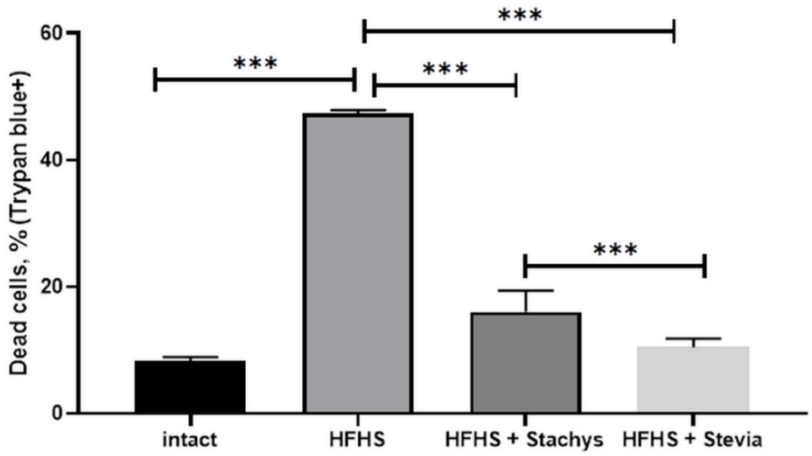

Figure 4. Viability indicators of bone marrow cells in immature rats in the experiment

Adding *Stachys* to the diet reduced the percentage of dead cells by 66.09 % ($p < 0.0001$) relative to HFHS, indicating a substantial protective effect. *Stevia* provided an even more pronounced reduction of 77.90 % ($p < 0.0001$) relative to HFHS, indicating stronger cytoprotective action in bone marrow (Fig. 4).

Discussions

Adding plant products to the HFHS diet differentially reshaped hematopoietic outcomes. Relative to HFHS alone, *Stevia* reduced the proportion of trypan-blue–positive cells by 53.45 % in the spleen and by 77.90 % in the bone marrow, indicating robust preservation of cell viability. HFHS by itself increased cell death and lowered nucleated-cell concentration in both tissues, consistent with diet-induced metabolic stress and inflammation. The pattern observed here is coherent with published evidence describing anti-inflammatory and anti-apoptotic actions of *Stevia*, including down-regulation of NF-κB/MAPK signaling [29]. Cai et al. (2023) noted *Stevia*'s ability to inhibit NF-κB and MAPK signaling pathways, associated with inflammation and apoptosis, leading to reduced extracellular matrix degradation and cell apoptosis [30].

Additionally, Gupta et al. (2021) demonstrated *Stevia*'s antioxidant and antidiabetic properties in rats with alloxan-induced diabetes. Oral administration of steviosides for 21 days normalized blood glucose levels, restored antioxidant potential, and improved lipid profiles. This indicates *Stevia*'s potential to improve metabolic status and reduce oxidative stress, supporting our observations of its cytoprotective effect [31].

Regarding the concentration of nucleated cells, *Stevia* increased their count in the spleen by 10.06 % compared to the HFHS group. Although this effect was less pronounced than that of *Stachys*, it still indicates a positive influence of *Stevia* on the immune system. Moubder et al. (2024) in their study noted that *Stevia* leaf extract increases levels of pro-inflammatory cytokine IL-1β and immunoglobulin A (IgA), indicating its immunomodulatory action [32].

On the other hand, *Stachys* showed a more pronounced effect on the proliferation of immune cells. In our study, adding *Stachys* to the HFHS diet increased the concentration of nucleated cells in the spleen by 35.55 % compared to the HFHS group. This may indicate stimulation of immune function and enhancement of immune response. However, in the bone marrow, *Stachys* further decreased the number of nucleated cells by 15.55 % compared to the HFHS group, suggesting a complex impact on hematopoiesis.

Studies by Kim et al. (2024) revealed that extracts of *Stachys affinis* possess antioxidant and anti-inflammatory properties confirmed by molecular docking. The phenolic compounds in the extract demonstrated the ability to interact with cyclooxygenase-2 (COX-2), reducing inflammation [33]. Slimani et al. (2023) found that *Stachys circinata* extract increases levels of antioxidant enzymes and exhibits cytotoxic effects on cancer cells, indicating its antiproliferative properties [34].

In terms of bone marrow cell viability, *Stachys* reduced the percentage of dead cells by 66.09 % relative to the HFHS group. Although this effect was less pronounced than that of *Stevia*, it still indicates a significant protective effect. Bayat et al. (2020) showed that *Stevia* increases the expression of antioxidant genes and improves kidney function in diabetic rats, which is consistent with our observation of its stronger cytoprotective action [35].

In summary, our data and findings from other studies emphasize the differences in the mechanisms of action of *Stevia* and *Stachys*. *Stevia rebaudiana* possesses strong cytoprotective effects, protecting cells from oxidative stress and apoptosis. This may be due to its ability to inhibit pro-inflammatory signaling pathways and increase antioxidant enzyme activity. *Stachys sieboldii*, on the other hand, stimulates immune cell proliferation, likely by influencing redox balance and modulating signaling pathways responsible for cell proliferation.

It is important to note that both plants exhibit protective properties against the negative effects of an HFHS diet, but with different emphases. This opens up the possibility for their combined use to achieve a more comprehensive therapeutic effect. For example, combining the cytoprotective effect of *Stevia* with the immunostimulatory effect of *Stachys* could provide more effective protection of the immune system and hematopoiesis from metabolic stress.

Conclusion

Metabolic disorders caused by unbalanced diets require an integrated approach to prevention and treatment. Energy-dense, unbalanced diets impair immune and hematopoietic compartments; prevention should therefore address both survival and renewal of cells. In our model, *Stevia* was superior for maintaining viability in both spleen and (particularly) bone marrow, whereas *Stachys* more effectively expanded the splenic pool of nucleated cells, consistent with immune-cell proliferation. Selection can thus be purpose-driven: *Stevia* when limiting cell death is the primary goal, and *Stachys* when the aim is to increase immune-cell numbers. A combined approach may be reasonable but warrants additional work on dosing, safety, and interactions. By delineating these complementary profiles, the study provides a basis for developing nutritional adjuncts and phytotherapeutic candidates to support hematopoiesis and immune function.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Pozdnyakova Ye.V.** — conceptualization, methodology, supervision, writing — original draft, writing — review & editing; **Murzatayeva A.M.** — investigation, data curation, formal analysis, visualization, writing — review & editing; **Sailau A.S.** — investigation, data curation, formal analysis, visualization, writing — original draft.

References

1 Goh E. V. Transforming Food Environments: A Global lens on challenges and opportunities for achieving healthy and sustainable diets for all / E. V. Goh, N. Sobratee-Fajurally, A. Allegretti, M. Sardeshpande, M. Mustafa, S. Azam-Ali, R. Omari, J. Schott, V. Chimonyo, D. Weible, G. Mutalemwa, T. Mabhaudhi, F. Massawe // Front. Sustain. Food Syst. — 2024. — Vol. 8. https://doi.org/10.3389/fsufs.2024.1366878

2 Clemente-Suárez V. J. Global impacts of western diet and its effects on metabolism and health: a narrative review / V. J. Clemente-Suárez, A. I. Beltrán-Velasco, L. Redondo-Flórez, A. Martín-Rodríguez, J. F. Tornero-Aguilera // Nutrients. — 2023. — Vol. 15, No. 12. — P. 2749. https://doi.org/10.3390/nu15122749

3 Wolfson J. A. Cooking at home, fast food, meat consumption, and dietary carbon footprint among US Adults / J. A. Wolfson, A. M. Willits-Smith, C. W. Leung, M. C. Heller, D. Rose // Int. J. Environ. Res. Public Health. — 2022. — Vol. 19, No. 2. — P. 853. https://doi.org/10.3390/ijerph19020853

4 Seale E. Fast food consumption in adults living in Canada: alternative measurement methods, consumption choices, and correlates / E. Seale, M. de Groh, L. Greene-Finestone // Appl. Physiol. Nutr. Metab. — 2023. — Vol. 48, No. 2. — P. 163–171. https://doi.org/10.1139/apnm-2022-0252

5 Baker P. Ultra-processed foods and the nutrition transition: global, regional, and national trends, food systems transformations and political economy drivers / P. Baker, P. Machado, T. Santos, K. Sievert., K. Backholer, M. Hadjikakou, C. Russell, O. Huse, C. Bell, G. Scrinis, A. Worsley, S. Friel, M. Lawrence // Obes. Rev. — 2020. — Vol. 21, No. 12. — P. e13126. https://doi.org/10.1111/obr.13126

6 Patel O. Association between full service and fast food restaurant density, dietary intake and overweight/obesity among adults in Delhi, India / O. Patel, S. Shahulhameed, R. Shivashankar, M. Tayyab, A. Rahman, D. Prabhakaran, N. Tandon, L. M. Jaacks // BMC Public Health. — 2017. — Vol. 18, No. 1. — P. 36. https://doi.org/10.1186/s12889-017-4598-8

7 Wu Y. Growing fast food consumption and obesity in Asia: challenges and implications / Y. Wu, L. Wang, J. Zhu, L. Gao, Y. Wang // Soc. Sci. Med. — 2021. — Vol. 269. — P. 113601. https://doi.org/10.1016/j.socscimed.2020.113601

8 Powell-Wiley T. M. Obesity and cardiovascular disease: a scientific statement from the American Heart Association / T. M. Powell-Wiley, P. Poirier, L. E. Burke, J. P. Després, P. Gordon-Larsen, C. J. Lavie, S. A. Lear, C. E. Ndumele, I. J. Neeland, P. Sanders, M. P. St-Onge // Circulation. — 2021. — Vol. 143, No. 21. — P. e984–e1010. https://doi.org/10.1161/CIR.0000000000000973

9 Koliaki C. Update on the obesity epidemic: after the sudden rise, is the upward trajectory beginning to flatten? / C. Koliaki, M. Dalamaga, S. Liatis // Curr. Obes. Rep. — 2023. — Vol. 12, No. 4. — P. 514–527. https://doi.org/10.1007/s13679-023-00527-y

10 Li Y. High fat diet-induced obesity exacerbates hematopoiesis deficiency and cytopenia caused by 5-fluorouracil via peroxisome proliferator-activated receptor γ / Y. Li, S. Zhu, Y. Zhang, T. Liu, L. Su, Q. Zhang, Y. Luo // Exp. Hematol. — 2018. — Vol. 60. — P. 30–39.e1. https://doi.org/10.1016/j.exphem.2017.12.013

11 Nelson B. N. Developmental programming of the fetal immune system by maternal western-style diet: mechanisms and implications for disease pathways in the offspring / B. N. Nelson, J. E. Friedman // Int. J. Mol. Sci. — 2024. — Vol. 25, No. 11. — P. 5951. https://doi.org/10.3390/ijms25115951

12 Carrillo-Martinez E. J. Quercetin, a flavonoid with great pharmacological capacity / E. J. Carrillo-Martinez, F. Y. Flores-Hernández, A. M. Salazar-Montes, H. F. Nario-Chaidez, L. D. Hernández-Ortega // Molecules. — 2024. — Vol. 29, No. 5. — P. 1000. https://doi.org/10.3390/molecules29051000

13 El-Kazaz S. E. The influence of quercetin on behavior, performance and splenic immunity in broiler chickens / S. E. El-Kazaz, M. H. Hafez, G. M. Albadrani, M. Q. Al-Ghadi, M. M. Abdel-Daim, Y. S. El-Sayed // Vet. Anim. Sci. — 2024. — Vol. 26. — P. 100398. https://doi.org/10.1016/j.vas.2024.100398

14 Farag M. R. Quercetin alleviates the immunotoxic impact mediated by oxidative stress and inflammation induced by doxorubicin exposure in rats / M. R. Farag, A. A. A. Moselhy, A. El-Mleeh, S. H. Aljuaydi, T. A. Ismail, A. Di Cerbo, G. Crescenzo, S. M. Abou-Zeid // Antioxidants. — 2021. — Vol. 10, No. 12. — P. 1906. https://doi.org/10.3390/antiox10121906

15 Khattab H. A. H. The Biological and Hematological Effects of Echinacea purpurea L. roots extract in the immunocompromised rats with cyclosporine / H. A. H. Khattab, S. K. Abounasef, H. L. Bakheet // J. Microsc. Ultrastruct. — 2019. — Vol. 7, No. 2. — P. 65–71. https://doi.org/10.4103/JMAU.JMAU_62_18

16 Sullivan A. M. Echinacea-induced macrophage activation / A. M. Sullivan, J. G. Laba, J. A. Moore, T. D. Lee // Immunopharmacol. Immunotoxicol. — 2008. — Vol. 30, No. 3. — P. 553–574. https://doi.org/10.1080/08923970802135534

17 Fu A. Echinacea purpurea extract polarizes M1 macrophages in murine bone marrow-derived macrophages through the activation of JNK / A. Fu, Y. Wang, Y. Wu, H. Chen, S. Zheng, Y. Li, X. Xu, W. Li // J. Cell. Biochem. — 2017. — Vol. 118, No. 9. — P. 2664–2671. https://doi.org/10.1002/jcb.25875

18 Rimmelé P. Resveratrol increases the bone marrow hematopoietic stem and progenitor cell capacity / P. Rimmelé, S. Lofek-Czubek, S. Ghaffari // Am. J. Hematol. — 2014. — Vol. 89, No. 12. — P. E235–E238. https://doi.org/10.1002/ajh.23837

19 Inchingolo A. D. Effects of resveratrol, curcumin and quercetin supplementation on bone metabolism – a systematic review / A. D. Inchingolo, A. M. Inchingolo, G. Malcangi, P. Avantario, D. Azzollini, S. Buongiorno, F. Viapiano, M. Campanelli // Nutrients. — 2022. — Vol. 14, No. 17. — P. 3519. https://doi.org/10.3390/nu14173519

20 Gao X. Immunomodulatory activity of resveratrol: suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity, and cytokine production / X. Gao, Y. X. Xu, N. Janakiraman, R.A. Chapman, S.C. Gautam // Biochem. Pharmacol. — 2001. — Vol. 62, No. 9. — P. 1299–1308. https://doi.org/10.1016/s0006-2952(01)00775-4

21 Tomou E. M. Genus Stachys: A review of traditional uses, phytochemistry and bioactivity / E. M. Tomou, C. Barda, H. Skaltsa // Medicines. — 2020. — Vol. 7, No. 10. — P. 63. https://doi.org/10.3390/medicines7100063

22 Benedec D. Stachys species: comparative evaluation of phenolic profile and antimicrobial and antioxidant potential / D. Benedec, I. Oniga, D. Hanganu, B. Tiperciuc, A. Nistor, A. M. Vlase, L. Vlase, C. Puşcaş, M. Duma, C. C. Login, M. Niculae, R. Silaghi-Dumitrescu // Antibiotics. — 2023. — Vol. 12, No. 11. — P. 1644. https://doi.org/10.3390/antibiotics12111644

23 Peteliuk V. Natural sweetener stevia rebaudiana: functionalities, health benefits and potential risks / V. Peteliuk, L. Rybchuk, M. Bayliak, K. B. Storey, O. Lushchak // EXCLI J. — 2021. — Vol. 20. — P. 1412–1430. https://doi.org/10.17179/excli2021-4211

24 Patel S. The Natural Sweetener Stevia: an updated review on its phytochemistry, health benefits, and anti-diabetic study / S. Patel, A. Navale // Curr. Diabetes Rev. — 2024. — Vol. 20, No. 2. — P. e010523216398. https://doi.org/10.2174/1573399819666230501210803

25 Ajami M. Effects of stevia on glycemic and lipid profile of type 2 diabetic patients: a randomized controlled trial / M. Ajami, M. Seyfi, F. Abdollah Pouri Hosseini, P. Naseri, A. Velayati, F. Mahmoudnia, M. Zahedirad, M. Hajifaraji // Avicenna J. Phytomed. — 2020. — Vol. 10, No. 2. — P. 118–127.

26 Гинс М. С. Стахис — перспективная овощная культура с лекарственными свойствами: биохимические и фармакологические свойства / М. С. Гинс, В. К. Гинс, П. Ф. Кононков // Овощи России. — 2015. — № 3(4). — С. 108–112. https://doi.org/10.18619/2072-9146-2015-3-4-108-112.

27 Hsieh M. H. Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: a two-year, randomized, placebo-controlled study / M. H. Hsieh, P. Chan, Y. M. Sue, J. C. Liu, T. H. Liang, T. Y. Huang, B. Tomlinson, M. S. Chow, P. F. Kao, Y. J. Chen // Clin. Ther. — 2003. — Vol. 25, No. 11. — P. 2797–2808. https://doi.org/10.1016/s0149-2918(03)80334-x

28 Полянская Г. Г. Методическое пособие по работе с клеточными культурами человека и животных / Г. Г. Полянская, Т. Н. Ефремова, А. М. Кольцова, А. С. Мусорина, Н. С. Шарлаимова, Т. К. Яковлева. — Санкт-Петербург: Политех-Пресс, 2019. — 114 с.

29 Othman F. Stevia extract safeguards rats from diabetic renal injury by mitigating markers of inflammation, oxidative stress, and apoptosis / F. Othman, M. El-Sawi, S. Shabana // Toxicol. Environ. Health Sci. — 2023. — Vol. 15. https://doi.org/10.1007/s13530-023-00188-3

30 Cai T. Stevioside targets the NF-κB and MAPK pathways for inhibiting inflammation and apoptosis of chondrocytes and ameliorates osteoarthritis in vivo / T. Cai, H. Ye, H. Jiang, C. Lin, C. Lou, W. Wang, Z. Yan, X. Xue, X. Pan, J. Lin // Int. Immunopharmacology. — 2023. — Vol. 115. — P. 109683. https://doi.org/10.1016/j.intimp.2023.109683

31 Gupta E. Antioxidant and anti-diabetic potential of rebaudioside A and a mixture of steviol glycosides in alloxan-induced diabetic rats / E. Gupta, A. Mohammed, N. Mishra, S. Purwar, I. Syed, S.I. Rizvi, S. Sundaram // Indian J. Nat. Prod. Resour. — 2021. — Vol. 12. — P. 391–399.

32 Moubder E. Immunomodulatory effects of Stevia rebaudiana leaves and commercial stevia on rats: a comparative study / E. Moubder, H. Abass, M. Al-Mahdawi, F. Abd // Rev. Clin. Pharmacol. Pharmacokinet. — 2024. — Vol. 38. — P. 153–156. https://doi.org/10.61873/IVJV6786

33 Kim H. H. Binding affinity screening of polyphenolic compounds in Stachys affinis extract (SAE) for their potential antioxidant and anti-inflammatory effects / H. H. Kim, S. H. Jeong, M. Y. Park, P. B. Bhosale, A. Abusaliya, S. J. Lee, J. D. Heo, H. W. Kim, J. K. Seong, D. I. Kim, K. I. Park, G. S. Kim // Sci. Rep. — 2024. — Vol. 14, No. 1. — P. 18095. https://doi.org/10.1038/s41598-024-68880-z

34 Slimani W. Antioxidant, anti-inflammatory and anti-proliferative properties of Stachys circinata on HepG2 and MCF7 cells / W. Slimani, M. Maioli, S. Cruciani, S. Zerizer, S. Santaniello, Z. Kabouche, D. Coradduzza, M. Chessa, S. Fancello, R. Migheli, P. A. Serr, G. D'hallewin // Plants. — 2023. — Vol. 12, No. 12. — P. 2272. https://doi.org/10.3390/plants12122272

35 Bayat E. Stevia rebaudiana extract attenuates metabolic disorders in diabetic rats via modulation of glucose transport and antioxidant signaling pathways and aquaporin-2 expression in two extrahepatic tissues / E. Bayat, Z. Rahpeima, S. Dastghaib, F. Gholizadeh, M. Erfani, G. Asadikaram, P. Mokarram // J. Food Biochem. — 2020. — Vol. 44, No. 8. — P. e13252. https://doi.org/10.1111/jfbc.13252

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Егеуқұйрықтардағы күйзеліс метаболизмі кезіндегі *Stachys sieboldii* және *Stevia rebaudiana* өсімдіктерінің цитопротекторлық және иммунитет стимулдеуші қасиеттері

Зерттеудің мақсаты майлар мен көмірсуларға бай диетамен (HFHS) тамақтандырылған егеуқұйрықтардағы *Stachys sieboldii* және *Stevia rebaudiana* сығындыларының сүйек кемігі мен көкбауыр жасушаларына қорғаныш әсерін бағалау. Зерттеудің негізгі бағыты осы өсімдіктердің цитопротекторлық және иммунитет стимулдеуші қасиеттерін зерттеу. Жұмыстың әдістемесі төрт топқа бөлінген, яғни жыныстық жетілмеген (өсімтал) Wistar егеуқұйрықтарына эксперименттер жүргізуді қамтыды. Атап айтсақ: бақылау тобы, HFHS тобы, *Stachys sieboldii* қосылған HFHS тобы және *Stevia rebaudiana* қосылған HFHS тобы. Көкбауыр мен сүйек кемігі жасушаларының жасушалық қасиеті мен тіршілік қабілеті 30 күн бойы бағаланды. Нәтижелер бойынша HFHS диета өлі жасушалардың көбеюіне және көкбауыр мен сүйек кемігіндегі ядросы бар жасушалардың концентрациясының төмендеуіне себеп болғанын көрсетті. *Stachys sieboldii* көкбауырдағы иммундық жасушалардың пролиферациясын айтарлықтай ынталандырып, ядросы бар жасушалардың концентрациясын арттырды, ал *Stevia rebaudiana* сүйек кемігіндегі өлі жасушалардың пайызын төмендете отырып, айқынырақ цитопротекторлық әсер көрсетті. Бұл зерттеудің құндылығы *Stachys sieboldii* және *Stevia rebaudiana* сығындыларының гемопоэтикалық жүйеге әртүрлі әсері және осы сығындыларды профилактикалық және иммундық қолдауға арналған қолданбаларда пайдалану мүмкіндігін ашады. Нәтижелердің практикалық маңыздылығы бұл өсімдіктерді биологиялық активті қоспалар құрамында, қантүзу және иммундық жүйелердің қалпын жақсарту үшін қолдануға болады.

Кілт сөздер: иммунитет, сүйек кемігі, көкбауыр, майға бай диета, HFHS, *Stevia rebaudiana, Stachys sieboldii*

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Цитопротекторные и иммуностимулирующие свойства *Stachys sieboldii* и *Stevia rebaudiana* в условиях метаболического стресса у крыс

Цель данного исследования — оценить протективные эффекты экстрактов *Stachys sieboldii* и *Stevia rebaudiana* на клетки костного мозга и селезёнки у крыс, получавших диету с высоким содержанием жиров и сахаров (HFHS). Мы проверили, способны ли экстракты *Stachys sieboldii* и *Stevia rebaudiana* противодействовать повреждениям, вызываемым диетой HFHS. Неполовозрелых крыс линии Wistar рандомизировали на четыре группы (контроль, HFHS, HFHS+*Stachys*, HFHS+*Stevia*) и содержали в течение 30 суток. Количественно оценивали клеточность селезёнки и костного мозга, а также жизнеспособность клеток. Диета HFHS увеличивала долю нежизнеспособных клеток и снижала концентрацию ядросодержащих клеток в обоих органах. *Stachys* главным образом усиливал пролиферацию иммунных клеток селезёнки (повышал концентрацию ядросодержащих клеток), тогда как *Stevia* вызывала более выраженный цитопротекторный ответ, уменьшая долю погибших клеток в костном мозге. В совокупности данные указывают на комплементарные действия — *Stachys* как пропролиферативный модулятор селезёнки и *Stevia* как протектор клеток кроветворной системы, что обосновывает целесообразность их рассмотрения в рамках стратегий питания для сохранения функций иммунной системы и кроветворения. Значимость данного исследования заключается в выявлении различающихся эффектов экстрактов *Stachys sieboldii* и *Stevia rebaudiana* на гемопоэтическую систему, что открывает потенциал их использования в профилактических и иммуноподдерживающих приложениях. Практическая ценность результатов состоит в возможности включения этих растений в состав диетических добавок для улучшения функции иммунной системы и кроветворения.

Ключевые слова: иммунитет, костный мозг, селезенка, высокожировая диета, HFHS, *Stevia rebaudiana*, *Stachys sieboldii*

References

1 Goh, E. V., Sobratee-Fajurally, N., Allegretti, A., Sardeshpande, M., Mustafa, M., Azam-Ali, S., Omari, R., Schott, J., Chimonyo, V., Weible, D., Mutalemwa, G., Mabhaudhi, T., & Massawe, F. (2024). Transforming food environments: A global lens on challenges and opportunities for achieving healthy and sustainable diets for all. *Frontiers in Sustainable Food Systems*, *8*. https://doi.org/10.3389/fsufs.2024.1366878

2 Clemente-Suárez, V. J., Beltrán-Velasco, A. I., Redondo-Flórez, L., Martín-Rodríguez, A., & Tornero-Aguilera, J. F. (2023). Global impacts of Western diet and its effects on metabolism and health: A narrative review. *Nutrients*, *15*(12), 2749. https://doi.org/10.3390/nu15122749

3 Wolfson, J. A., Willits-Smith, A. M., Leung, C. W., Heller, M. C., & Rose, D. (2022). Cooking at home, fast food, meat consumption, and dietary carbon footprint among US adults. *International Journal of Environmental Research and Public Health*, *19*(2), 853. https://doi.org/10.3390/ijerph19020853

4 Seale, E., de Groh, M., & Greene-Finestone, L. (2023). Fast food consumption in adults living in Canada: Alternative measurement methods, consumption choices, and correlates. *Applied Physiology, Nutrition, and Metabolism*, *48*(2), 163–171. https://doi.org/10.1139/apnm-2022-0252

5 Baker, P., Machado, P., Santos, T., Sievert, K., Backholer, K., Hadjikakou, M., Russell, C., Huse, O., Bell, C., Scrinis, G., Worsley, A., Friel, S., & Lawrence, M. (2020). Ultra-processed foods and the nutrition transition: Global, regional, and national trends, food systems transformations and political economy drivers. *Obesity Reviews*, *21*(12), e13126. https://doi.org/10.1111/obr.13126

6 Patel, O., Shahulhameed, S., Shivashankar, R., Tayyab, M., Rahman, A., Prabhakaran, D., Tandon, N., & Jaacks, L. M. (2017). Association between full service and fast food restaurant density, dietary intake and overweight/obesity among adults in Delhi, India. *BMC Public Health*, *18*(1), 36. https://doi.org/10.1186/s12889-017-4598-8

7 Wu, Y., Wang, L., Zhu, J., Gao, L., & Wang, Y. (2021). Growing fast food consumption and obesity in Asia: Challenges and implications. *Social Science & Medicine*, *269*, 113601. https://doi.org/10.1016/j.socscimed.2020.113601

8 Powell-Wiley, T. M., Poirier, P., Burke, L. E., Després, J.-P., Gordon-Larsen, P., Lavie, C. J., Lear, S. A., Ndumele, C. E., Neeland, I. J., Sanders, P., & St-Onge, M.-P. (2021). Obesity and cardiovascular disease: A scientific statement from the American Heart Association. *Circulation*, *143*(21), e984–e1010. https://doi.org/10.1161/CIR.0000000000000973

9 Koliaki, C., Dalamaga, M., & Liatis, S. (2023). Update on the obesity epidemic: After the sudden rise, is the upward trajectory beginning to flatten? *Current Obesity Reports*, *12*(4), 514–527. https://doi.org/10.1007/s13679-023-00527-y

10 Li, Y., Zhu, S., Zhang, Y., Liu, T., Su, L., Zhang, Q., & Luo, Y. (2018). High-fat diet-induced obesity exacerbates hematopoiesis deficiency and cytopenia caused by 5-fluorouracil via peroxisome proliferator-activated receptor γ. *Experimental Hematology*, *60*, 30–39.e1. https://doi.org/10.1016/j.exphem.2017.12.013

11 Nelson, B. N., & Friedman, J. E. (2024). Developmental programming of the fetal immune system by maternal Western-style diet: Mechanisms and implications for disease pathways in the offspring. *International Journal of Molecular Sciences*, *25*(11), 5951. https://doi.org/10.3390/ijms25115951

12 Carrillo-Martinez, E. J., Flores-Hernández, F. Y., Salazar-Montes, A. M., Nario-Chaidez, H. F., & Hernández-Ortega, L. D. (2024). Quercetin, a flavonoid with great pharmacological capacity. *Molecules*, *29*(5), 1000. https://doi.org/10.3390/molecules29051000

13 El-Kazaz, S. E., Hafez, M. H., Albadrani, G. M., Al-Ghadi, M. Q., Abdel-Daim, M. M., & El-Sayed, Y. S. (2024). The influence of quercetin on behavior, performance and splenic immunity in broiler chickens. *Veterinary and Animal Science, 26*, 100398. https://doi.org/10.1016/j.vas.2024.100398

14 Farag, M. R., Moselhy, A. A. A., El-Mleeh, A., Aljuaydi, S. H., Ismail, T. A., Di Cerbo, A., Crescenzo, G., & Abou-Zeid, S. M. (2021). Quercetin alleviates the immunotoxic impact mediated by oxidative stress and inflammation induced by doxorubicin exposure in rats. *Antioxidants*, *10*(12), 1906. https://doi.org/10.3390/antiox10121906

15 Khattab, H. A. H., Abounasef, S. K., & Bakheet, H. L. (2019). The biological and hematological effects of *Echinacea purpurea* L. roots extract in immunocompromised rats with cyclosporine. *Journal of Microscopy and Ultrastructure*, *7*(2), 65–71. https://doi.org/10.4103/JMAU.JMAU_62_18

16 Sullivan, A. M., Laba, J. G., Moore, J. A., & Lee, T. D. (2008). Echinacea-induced macrophage activation. *Immunopharmacology and Immunotoxicology*, *30*(3), 553–574. https://doi.org/10.1080/08923970802135534

17 Fu, A., Wang, Y., Wu, Y., Chen, H., Zheng, S., Li, Y., Xu, X., & Li, W. (2017). *Echinacea purpurea* extract polarizes M1 macrophages in murine bone marrow-derived macrophages through the activation of JNK. *Journal of Cellular Biochemistry*, *118*(9), 2664–2671. https://doi.org/10.1002/jcb.25875

18 Rimmelé, P., Lofek-Czubek, S., & Ghaffari, S. (2014). Resveratrol increases the bone marrow hematopoietic stem and progenitor cell capacity. *American Journal of Hematology*, *89*(12), E235–E238. https://doi.org/10.1002/ajh.23837

19 Inchingolo, A. D., Inchingolo, A. M., Malcangi, G., Avantario, P., Azzollini, D., Buongiorno, S., Viapiano, F., & Campanelli, M. (2022). Effects of resveratrol, curcumin and quercetin supplementation on bone metabolism – a systematic review. *Nutrients*, *14*(17), 3519. https://doi.org/10.3390/nu14173519

20 Gao, X., Xu, Y. X., Janakiraman, N., Chapman, R. A., & Gautam, S. C. (2001). Immunomodulatory activity of resveratrol: Suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity, and cytokine production. *Biochemical Pharmacology*, *62*(9), 1299–1308. https://doi.org/10.1016/S0006-2952(01)00775-4

21 Tomou, E. M., Barda, C., & Skaltsa, H. (2020). Genus *Stachys*: A review of traditional uses, phytochemistry and bioactivity. *Medicines*, *7*(10), 63. https://doi.org/10.3390/medicines7100063

22 Benedec, D., Oniga, I., Hanganu, D., Tiperciuc, B., Nistor, A., Vlase, A. M., Vlase, L., Puşcaş, C., Duma, M., Login, C. C., Niculae, M., & Silaghi-Dumitrescu, R. (2023). *Stachys* species: Comparative evaluation of phenolic profile and antimicrobial and antioxidant potential. *Antibiotics*, *12*(11), 1644. https://doi.org/10.3390/antibiotics12111644

23 Peteliuk, V., Rybchuk, L., Bayliak, M., Storey, K. B., & Lushchak, O. (2021). Natural sweetener *Stevia rebaudiana*: Functionalities, health benefits and potential risks. *EXCLI Journal*, *20*, 1412–1430. https://doi.org/10.17179/excli2021-4211

24 Patel, S., & Navale, A. (2024). The natural sweetener stevia: An updated review on its phytochemistry, health benefits, and anti-diabetic study. *Current Diabetes Reviews*, *20*(2), e010523216398. https://doi.org/10.2174/1573399819666230501210803

25 Ajami, M., Seyfi, M., Abdollah Pouri Hosseini, F., Naseri, P., Velayati, A., Mahmoudnia, F., Zahedirad, M., & Hajifaraji, M. (2020). Effects of stevia on glycemic and lipid profile of type 2 diabetic patients: A randomized controlled trial. *Avicenna Journal of Phytomedicine*, *10*(2), 118–127.

26 Gins, M. S., Gins, V. K., & Kononkov, P. F. (2015). Stakhis — perspektivnaia ovoshchnaia kultura s lekarstvennymi svoistvami: biokhimicheskie i farmakologicheskie svoistva [*Stachys* is a promising vegetable crop with officinal properties: Biochemical and pharmacological properties]. *Ovoshchi Rossii — Vegetable crops of Russia*, 3(4), 108–112. https://doi.org/10.18619/2072-9146-2015-3-4-108-112 [in Russian].

27 Hsieh, M.-H., Chan, P., Sue, Y.-M., Liu, J.-C., Liang, T.-H., Huang, T.-Y., Tomlinson, B., Chow, M. S., Kao, P.-F., & Chen, Y.-J. (2003). Efficacy and tolerability of oral stevioside in patients with mild essential hypertension: A two-year, randomized, placebo-controlled study. *Clinical Therapeutics*, *25*(11), 2797–2808. https://doi.org/10.1016/S0149-2918(03)80334-X

28 Polianskaia, G. G., Efremova, T. N., Koltsova, A. M., Musorina, A. S., Sharlaimova, N. S., & Yakovleva, T. K. (2019). *Metodicheskoe posobie po rabote s kletochnymi kulturami cheloveka i zhivotnykh* [Methodological Handbook for Working with Human and Animal Cell Cultures]. Saint Peterburg: Politekh-Press [in Russian].

29 Othman, F., El-Sawi, M., & Shabana, S. (2023). Stevia extract safeguards rats from diabetic renal injury by mitigating markers of inflammation, oxidative stress, and apoptosis. *Toxicological and Environmental Health Sciences, 15*. https://doi.org/10.1007/s13530-023-00188-3

30 Cai, T., Ye, H., Jiang, H., Lin, C., Lou, C., Wang, W., Yan, Z., Xue, X., Pan, X., & Lin, J. (2023). Stevioside targets the NF-κB and MAPK pathways for inhibiting inflammation and apoptosis of chondrocytes and ameliorates osteoarthritis *in vivo*. *International Immunopharmacology*, *115*, 109683. https://doi.org/10.1016/j.intimp.2023.109683

31 Gupta, E., Mohammed, A., Mishra, N., Purwar, S., Syed, I., Rizvi, S. I., & Sundaram, S. (2021). Antioxidant and anti-diabetic potential of rebaudioside A and a mixture of steviol glycosides in alloxan-induced diabetic rats. *Indian Journal of Natural Products and Resources*, *12*, 391–399.

32 Moubder, E., Abass, H., Al-Mahdawi, M., & Abd, F. (2024). Immunomodulatory effects of *Stevia rebaudiana* leaves and commercial stevia on rats: A comparative study. *Reviews in Clinical Pharmacology and Pharmacokinetics*, *38*, 153–156. https://doi.org/10.61873/IVJV6786

33 Kim, H. H., Jeong, S. H., Park, M. Y., Bhosale, P. B., Abusaliya, A., Lee, S. J., Heo, J. D., Kim, H. W., Seong, J. K., Kim, D. I., Park, K. I., & Kim, G. S. (2024). Binding affinity screening of polyphenolic compounds in *Stachys affinis* extract (SAE) for their potential antioxidant and anti-inflammatory effects. *Scientific Reports*, *14*(1), 18095. https://doi.org/10.1038/s41598-024-68880-z

34 Slimani, W., Maioli, M., Cruciani, S., Zerizer, S., Santaniello, S., Kabouche, Z., Coradduzza, D., Chessa, M., Fancello, S., Migheli, R., Serra, P. A., & D'Hallewin, G. (2023). Antioxidant, anti-inflammatory and anti-proliferative properties of *Stachys circinata* on HepG2 and MCF7 cells. *Plants*, *12*(12), 2272. https://doi.org/10.3390/plants12122272

35 Bayat, E., Rahpeima, Z., Dastghaib, S., Gholizadeh, F., Erfani, M., Asadikaram, G., & Mokarram, P. (2020). *Stevia rebaudiana* extract attenuates metabolic disorders in diabetic rats via modulation of glucose transport and antioxidant signaling pathways and aquaporin-2 expression in two extrahepatic tissues. *Journal of Food Biochemistry*, *44*(8), e13252. https://doi.org/10.1111/jfbc.13252

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Age-related adaptation features of teachers of educational institutions

Modern educational reforms require teachers to be highly qualified and to adapt quickly to new working methods, including the use of digital technologies. The problem of teachers' professional adaptation remains relevant despite a large number of studies in this field. Aim: to determine the factors influencing the professional adaptation of teachers in educational institutions, as well as to analyze the age characteristics of adaptation and the psychophysiological characteristics of teachers. The study involved 137 teachers from the city of Karaganda, who were divided into three age groups: under 30 years, 30–45 years and over 45 years. The Spielberger–Khanin test was used to assess anxiety, the WAM test was applied for subjective assessment of well-being, activity and mood, the Anfimov's correction tables were used to study mental performance, as well as physiological methods (blood pressure measurement, analysis of heart rate variability). The level of reactive anxiety among teachers did not show significant differences between the groups. However, the analysis of well-being, activity, and mood showed that well-being decreased with age, activity levels showed slight fluctuations, and mood was higher in the first group. Mental performance also decreased with age, which was confirmed by the decrease in the number of viewed and found signs. In addition, an increase in blood pressure, heart rate, and the stress index of regulatory systems was observed. Higher activity of the sympathetic nervous system and pronounced functional tension of regulatory systems were also identified in the older groups. The results of the study confirm the need to support teachers at different stages of their professional activities. Measures aimed at reducing stress load and increasing teachers' level of adaptation in the context of the digital transformation of education are particularly important.

Keywords: teachers of educational institutions, age dynamics, adaptation, psychophysiological state, working capacity.

Introduction

Improving of pedagogical methods, including the development of inclusive education, as well as the new work tools integration such as electronic journals and diaries, modern information technologies, etc., requires teachers to be highly qualified and capable of rapid adaptation [1-2]. In such conditions, the problem of professional adaptation of teachers becomes particularly relevant, since the problem of securing and retaining of teachers in educational institutions is not being solved, despite a sufficient number of studies [3].

The activity of a teacher in the process of professional development involves overcoming certain crisis states and adaptation [4-5]. The works emphasize the need to create a favorable educational environment that supports and stimulates the professional growth of teachers.

The neuropsychiatric load of teachers, which has increased as a result of the reform of the education system, is associated with the risk of somatic and mental disorders [6-7]. At the same time, psychosocial factors of production, especially those related to work organization, have a greater impact on mental health than on physical [8–10].

Professional health risk factors for teachers include such specific factors characteristic of educational activities as high responsibility, irregular working hours, including out-of-hours action, significant requirements for the assimilation, preservation and reproduction of information, heavy loads on the vocal apparatus and additional loads (checking students' written papers and preparing for lessons, etc.) [11-12, etc.]. It is necessary to single out particularly specific factors, namely intense psychoemotional loads, which are also characteristic of this professional category, the presence of which largely determines deviations in the health status of teachers [13-14].

According to the data [15-16], work intensity is the leading unfavorable factor in the work process of teachers. According to the respondents, work exhausts teachers not only physically (about 80 %), but also

morally (almost 90 %). The professional activity of teachers is characterized by high emotional workload. With professional experience increasing, most teachers begin to feel the "pedagogical crisis", "exhaustion", and "burnout", which significantly affects their mental and physical health [17]. Therefore, the study of these mechanisms is of great importance for optimizing the functioning of teachers in changing conditions, which makes it possible to increase the effectiveness of their activities and ensure a better quality of life [18]. However, the research does not fully reveal the specifics of the age-related adaptation of teachers to the conditions of professional activity, the psychological characteristics that ensure the success of the adaptation process, and most importantly, the effectiveness of professional adaptation. In this regard, the study of the problem of teachers' adaptation to professional activity in the age aspect is still relevant.

Experimental

The article presents the results of the study conducted among 137 teachers of secondary schools in Karaganda city. The teachers were distributed by age in the following groups: group I — 18 persons under the age of 30 (average age 25.7±0.52), group II — 65 persons at the age of 30–45 (average age 37.6±0.55), group III — 54 persons over the age of 45 (average age 52.7±0.7).

The sample was made up of teachers from general education institutions, including secondary schools and gymnasiums. The study involved teachers of primary, basic, and secondary general education who teach humanities, natural sciences, and mathematics. Since participation in the survey was voluntary and guaranteed complete anonymity, no separate written consent was requested. By returning the completed questionnaire, the participant confirmed his informed consent to participate in the study and gave permission to process the provided data. The study was approved by the Local Bioethics Commission of Karaganda Medical University (Protocol No. 17 dated 10/22/2024) and the Department of Education of the city of Karaganda in accordance with all the norms of the Helsinki Declaration (2013).

To determine the level of reactive anxiety, the Spielberger test in the modification of Khanin was used in the form of the questionnaire [19]. The results were evaluated on the following scale: up to 30 points — low anxiety, from 31 to 44 points — average, 45 points and above — high anxiety.

The subjective state of teachers was assessed by the results of the WAM test proposed by V.A. Doskin and his colleagues in 1973 [20]. This test covered three main characteristics: well-being, activity, and mood (WAM). With the WAM test, it is possible to identify specific aspects of the teachers' activities and condition, including their mood, concentration, stress level and efficiency. The questionnaire consisted of 30 pairs of characteristics that had opposite values. The analysis results were grouped according to the predefined key within three categories, and then the mean scores for each of them were calculated.

Blood pressure and heart rate (pulse) were used to assess the state of the cardiovascular system. Systolic and diastolic blood pressure was measured using the Korotkov–Yanovsky method while the subject was in the sitting position. The pulse was calculated by palpation in 60 seconds in the same position of subject.

To determine the level and dynamics of mental performance, proofreading tests for one minute were conducted using letter tables developed by V. Ya. Anfimov [21]. At the end of the test, the number of viewed and found letters was calculated, as well as the number of errors made during the task (these could be missing or incorrectly marked letters). We also calculated the attention intensity (AI) as the percentage of the number of viewed letters (VL) to the total number of letters (TL = 1600). The formula for AI calculating is as follows: **AI = VL / TL * 100**. Thus, when analyzing the test results, it was possible to get the understanding of the degree of concentration and efficiency of the subjects.

The analysis of heart rate variability (HRV) was performed by 5-minute cardiointervalography using the Varikard-2.4 software package [22]. The following statistical parameters of the heart rhythm were analyzed: the mean RR interval (mathematical expectation—Mean), the stress index (stress index—SI) of regulatory systems, characterizing the degree of centralization of heart rhythm control. The relative activity of the subcortical sympathetic nerve center was assessed by the ratio of the mean values of the low-frequency and high-frequency HRV components (LF/HF). The activity of the heart rate regulatory systems was assessed by the centralization index (CI = TP/HF), the level of activation of subcortical nerve centers was assessed by the calculated value characterizing the activity of regulatory systems (indicator of the activity of the regulatory systems—IRSA) [23].

The statistical analysis of the study results was carried out using the standard MS Microsoft Excel 2019 and STASTICA 10.0 software packages and included the calculation of the mean value of the variable (M)

and its standard error (±m). Intergroup differences between age categories were analyzed using the Student's criterion (t), which revealed statistically significant trends and patterns. The differences between the age groups were considered significant at $p<0.05$.

Results and Discussion

As the results of the study showed, the level of reactive anxiety among teachers of secondary schools was on average: in group I — 42±1.92 units, in group II — 42.5±1.24 units, in group III — 42.7±1.25 units (Fig. 1). No significant differences were found between the groups.

The analysis of the percentage of well-being, activity, and mood levels revealed the following dynamics. The well-being index in group I was 5.06±0.174 units, in group II — 4.62±0.147 units, in group III — 4.42±0.152 units ($p<0.05$). There were no significant differences in the activity index: in group I — 3.96±0.251 units, in group II — 3.67±0.141 units, in group III — 3.78±0.147 units. The dynamics of the mood index was studied, and it showed the following values: in group I — 5.9±0.317 units, in group II — 4.84±0.156 units, in group III — 4.93±0.145 units, however, there were no statistically significant differences.

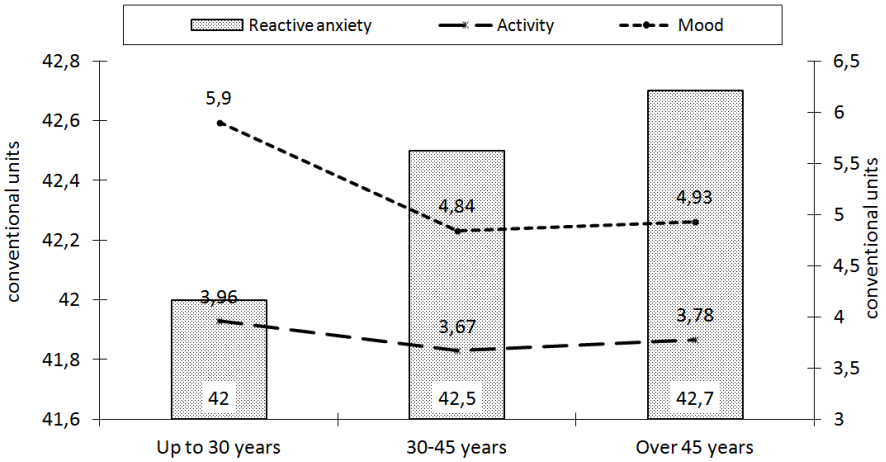

Figure 1. Age-related dynamics of reactive anxiety and WAM indicators

When evaluating the proofreading tests according to the table of V. Ya. Anfimov, significant differences in mental performance were revealed (Fig. 2). The average number of viewed letters in group I was 435.7±25.86, in group II — 415.4±20.72, in group III — 373.7±18.45 ($p<0.05$). The number of found letters in group I was 51.5±3.15, in group II — 49.9±2.24, in group III — 43.8±2.12 ($p<0.05$). The dynamics of the attention index showed the highest values in group I — 27.2±1.62, in group II it was 25.9±1.29, in group III — 23.3±1.15 ($p<0.05$). The number of errors had no significant differences and amounted to 0.7±0.23 in group I, 1±0.19 — in group II, and 0.6±0.19 characters — in group III.

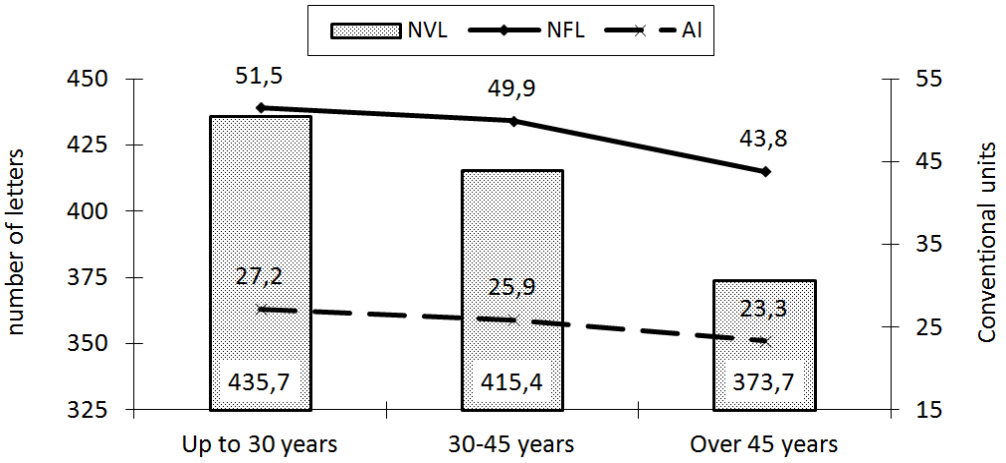

Figure 2. Age-related dynamics of indicators of correction tests according to the table of V. Ya. Anfimov

The analysis of blood pressure indicators revealed their significant increase in age dynamics (Fig. 3). The mean value of diastolic pressure in group I was 73±3.21 mmHg, in group II — 82.3±1.92 mmHg ($p<0.05$), in group III — 93.7±2.45 mmHg. ($p<0.05$). The mean value of systolic blood pressure in group I was 106.6±3.35 mmHg, in group II — 117.7±2 mmHg ($p<0.05$), in group III — 127.4±2.04 mmHg ($p<0.05$). The heart rate also showed the significant increase: in group I — 74.1±1.18 beats/min, in group II — 78.6±0.83 beats/min ($p<0.05$), in group III — 80.3±1 beats/min ($p<0.05$).

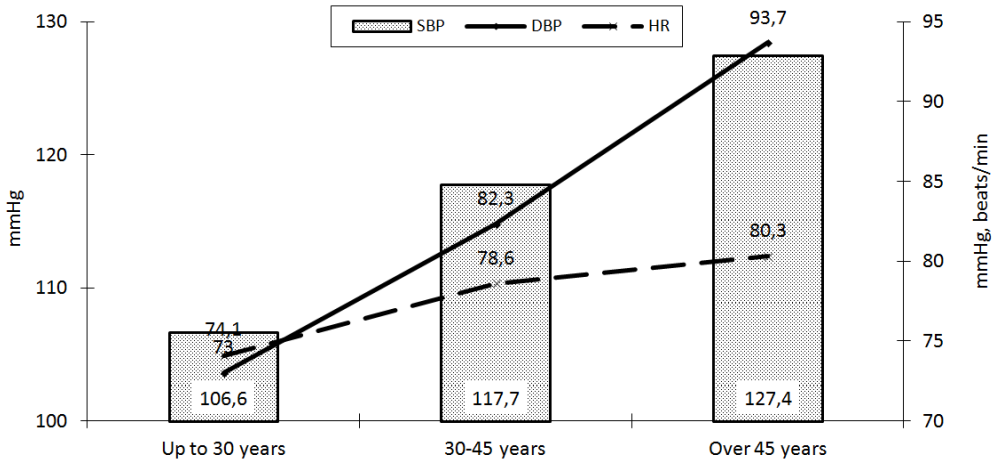

Figure 3. Age-related dynamics of indicators of the cardiovascular system functioning

The mean value of the stress index (SI) of regulatory systems in dynamics was 352.4±100.83 and 352.5±85.72 units in groups I and II, respectively, in group III the indicator increased to 401.2±62.53 units (Fig. 4). The ratio of the mean values of the low-frequency and high-frequency components of HRV (LF/HF) was 1.3±0.27 units in group I, 3±0.62 units — in group II ($p<0.05$), and 3.8±0.81 units — in group III ($p<0.05$), indicating increased activity of the subcortical sympathetic nerve center. The heart rate control centralization index (CI) in group I was 2.4±0.52 units, in group II — 5.2±0.98 units ($p<0.05$), in group III — 6.4±1.21 units ($p<0.05$). Analysis of the dynamics of the indicator of regulatory systems activity (IRSA) showed that in group I it corresponded to the "expressed functional stress" level (4.2±0.34 units), in groups II and III it increased to the "strongly expressed functional stress" level and amounted to 5.1±0.3 and 5.2±0.41 units, respectively.

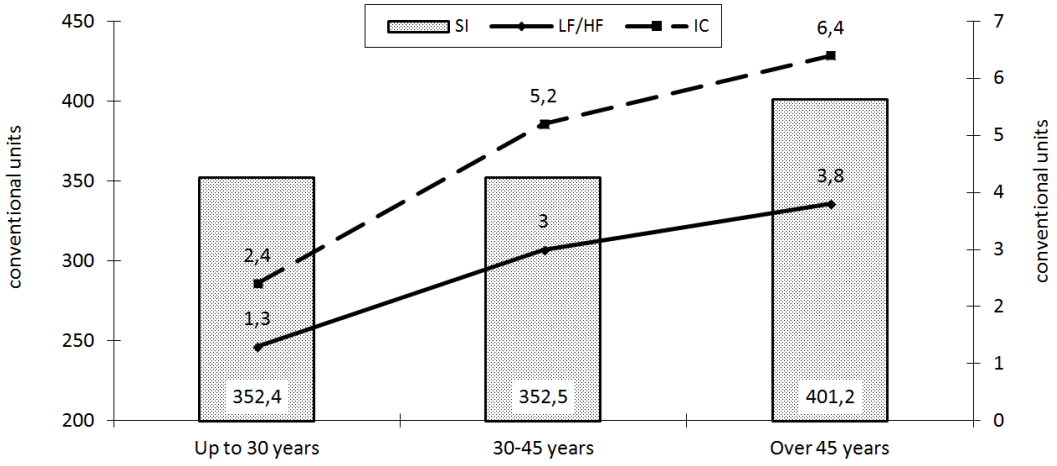

Figure 4. Age-related dynamics of heart rate variability indicators

The intensity of the teacher's work is determined by several factors reflecting mental stress: creative approach to tasks, analysis, assessment and observation of the learning process, activities with limited time, emotional involvement and responsibility for the result of work, the occurrence of controversial situations related to the profession, sensory overload and lack of physical activity [24].

Pedagogical activity requires the teacher to constantly interact with students, colleagues, and the administration, and it creates increased demands on the adaptive capabilities of the body. Studies show that teachers have the increased level of psychoemotional stress, which eventually leads to the depletion of adaptive mechanisms [25].

It was noted [26] that age-related crises among teachers aged 40–45 years are accompanied by the job satisfaction decrease in and the anxiety increase, which can manifest in the deterioration of well-being and cognitive functions. This corresponds to the data of our study, which revealed the decrease in the indicators of well-being and activity among teachers of older age groups.

According to the study of the European Occupational Safety and Health Agency (EU-OSHA, 2021), teachers are at the increased risk of occupational stress developing, which leads to the development of psychosomatic diseases [27-28].

The number of studies [29-30] demonstrate that teachers have increased levels of cortisol, a stress hormone, which confirms the influence of professional activity on the endocrine system. The results of our study also indicate the increase in sympathetic activity (LF/HF increase) with age, which indicates the increasing effect of stress.

Psychophysiological stress leads to functional changes in the body, such as the deterioration in well-being and subjective assessment of the condition (according to our data, this is the decrease in well-being, activity and mood), the cognitive functions decrease (decrease in the number of viewed and found letters, the attention index), high blood pressure and heart rate (our study revealed the increase in systolic and diastolic blood pressure and heart rate with age).

Prolonged exposure to stress without adequate compensatory mechanisms leads to the development of professional burnout syndrome [31-32]. Chronic stress among teachers leads to increased fatigue and decreased performance, sleep disorders, depressive states and, as a result, the development of cardiovascular diseases [33-34].

These data are confirmed by the results of our study, which revealed the dynamics of deterioration in the indicators of regulatory systems, which indicates a gradual depletion of adaptive resources.

Studies of the psychophysiological state of teachers indicate the high workload associated with their professional activities. The work of a teacher requires not only high cognitive activity, but also significant emotional and communicative costs, which leads to the formation of chronic stress and tension of adaptive processes [35-36]. In general, the results of the study emphasize the need to develop comprehensive programs aimed at maintaining the psychophysiological health of teachers of various age groups, taking into account specific changes related to age and professional activity.

Conclusion

1. Stress caused by professional activity has the expressed effect on cognitive functions, which is manifested by the mental performance and attention decrease. With the years, teachers experience the decrease in the amount of the processed information and the decrease in the attention index, which may affect the quality of teaching and require the development of support programs and trainings to maintain cognitive functions.

2. The adaptive mechanisms of teachers have significant stress, which is expressed in the physiological stress markers increase (increased blood pressure, stress index of regulatory systems and activity of the sympathetic nervous system). Long-term exposure to stress can lead to serious health consequences, namely the development of hypertension, cardiovascular diseases, emotional burnout and mental disorders.

3. In order to minimize the effects of professional stress on teachers, it is necessary to monitor the psychophysiological state of teachers and develop adaptation strategies, increase the stress tolerance of this category of specialists through special trainings and self-regulation programs.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Smagulov N.K. —** Conception and design of study, methodology, Analysis and/or interpretation of data, supervision, writing draft, editing. **Arystanbay A.A. —** data curation, formal analysis, investigation, Drafting the manuscript. **Tykezhanova G.M. —** data curation, formal analysis, investigation. **Svetlik M.V. —** methodology, Analysis and/or interpretation of data, writing draft, editing.

References

1 Куркина Н.Р. Профессиональная адаптация молодых педагогов в условиях цифровой трансформации образования / Н.Р. Куркина, Л.Н. Потапова, С.М. Имяреков / Н.Р. Куркина, Л.Н. Потапова, С.М. Имяреков // Современные проблемы науки и образования. — 2024. — № 6.

2 Шавалеева Г.М. Роль учителя в школе и в обществе / Г.М. Шавалеева // Отечественная и зарубежная педагогика. — 2022. — № 4. — С. 119–124.

3 Дадаева М.С. Профессиональная адаптация педагогов как ключевая задача кадрового менеджмента образовательной организации / М.С. Дадаева, З.С. Магомадова, М.М. Минкаилова // Индустриальная экономика. — 2022. — № 3. — С. 240–243.

4 Котова С.А. Адаптация в должности и освоение профессии учителя / С.А. Котова // Народное образование. — 2010. — № 8. — С. 121–127.

5 Садовникова Н.О. Профессиональный кризис личности педагога: содержание и основные признаки / Н.О. Садовникова // Научный диалог. — 2016. — № 11 (59). — С. 400–411.

6 Мишкич И.А. Влияние нервно-эмоциональной напряжённости трудового процесса на организм педагогических и медицинских работников / И.А. Мишкич, Е.А. Баймаков, О.И. Юшкова // Медицина труда и промышленная экология — 2021. — Т. 61, № 4. — С. 218–223.

7 Bomfim M. Teacher education in health: assessment, issues and tensions / M. Bomfim, V. Goulart, L. de Oliveira // Comunicação saúde educação. — 2014. — Vol. 18. — P. 749–758.

8 Litchfield P. Work and Wellbeing in the 21st Century / P. Litchfield, C. Cooper, Ch. Hancock, P. Watt // Int. J. Environ. Res. Public. Health. — 2016. — Vol. 13, No. 11. — P. 1065.

9 Corrente M. Mental Health Experiences of Teachers: A Scoping Review / M. Corrente, K. Ferguson, I. Bourgeault // Journal of Teaching and Learning. — 2022. — Vol. 16, No. 1. — P. 22–43.

10 Azarov T.A. Health in the system of teachers' values: health-saving behavior and lifestyle / T.A. Azarov, M.S. Mikerova, N.A. Sokolov // Public Health. — 2024. — No. 4(4). — P. 16–23.

11 Багнетова Е.А. Профессиональные риски педагогической среды / Е.А. Багнетова, Е.Р. Шарифуллина // Фундаментальные исследования. — 2013. — № 1 (часть 1). — С. 27–31.

12 Pecherkina A. Features of Professional Health of a Teacher at Different Stages of Professional Development / A. Pecherkina, R.Muslumov // The Fifth International Luria Memorial Congress "Lurian Approach in International Psychological Science". — Ekaterinburg, 2018. — P. 700–710.

13 Кулганов В. Психоэмоциональное напряжение и утомление учителя: механизмы, диагностика и профилактика / В. Кулганов // Известия Российского государственного педагогического университета им. А.И. Герцена. — 2009. — № 100. — С. 131–142.

14 Шабас С.Г. Психофизическое здоровье современного преподавателя как условие качества образовательного процесса в российской высшей школе / С.Г. Шабас // Мир науки. Педагогика и психология. — 2023. — Т. 11, № 4. — С. 1–15.

15 Степанов Е.Г. Труд и здоровье учителей общеобразоватльных школ в современных условиях / Е.Г. Степанов, Р.М. Фасиков, Н.А. Диденко // Медицина труда и промышленная экология. — 2010. — № 6. — С. 45–49.

16 Yob F. The Factors Affecting School Teachers' Wellbeing during Pandemic Era: A Scoping Review / F. Yob, A. Ghani, L. Pek, et al. // Open access. — 2022. — Vol. 11, No. 4. — P. 887–904.

17 Панков В.А. Условия труда и состояние здоровья педагогов общеобразовательных организаций (обзор литературы) / В.А. Панков, Е.В. Катаманова, Н.В. Сливницына // Гигиена и санитария. — 2022. — Т. 101, № 8. — С. 940–946.

18 Черникова Е.Г. Особенности социально-профессиональной адаптации молодых педагогов общеобразовательных школ / Е.Г. Черникова // Вестник Челябинского государственного университета. — 2014. — № 4 (333). — Вып. 3. — С. 150–153.

19 Ханин Ю.Л. Краткое руководство к шкале реактивной и личностной тревожности Ч.Д. Спилбергера / Ю.Л. Ханин // Ленинград. — 1976. — 18 с.

20 Доскин В.А. Тест дифференцированной самооценки функционального состояния / В.А. Доскин, Н.А. Лаврентьева, М.П. Мирошников, В.Б. Шарай // Вопросы психологии. —1973. — № 6. — С. 141.

21 Кузнецов А.П. Исследование умственной работоспособности / А.П. Кузнецов, Л.Н. Смелышева, Н.В. Сажина, О.А. Архипова // Методические рекомендации к проведению практических и лабораторных занятий для студентов направлений подготовки. Изд-во КГУ. — Курган, 2019. — 43 с.

22 Шлык Н.И. Вариабельность сердечного ритма у спортсменов в тренировочном процессе: методическое пособие / Н.И. Шлык. — Ижевск: Удмуртский университет, 2022. — 80 с.

23 Баевский Р.М. Анализ вариабельности сердечного ритма: физиологические основы и основные методы проведения / Р.М. Баевский, А. Черникова // Cardiometry. — 2017. — Т. 10. — С. 68–80.

24 Кучма В.Р. Гигиеническая оценка образа жизни, напряжённости и режима труда, состояния здоровья и психологического благополучия педагогов / В.Р. Кучма, С.Б. Соколова, И.К. Рапопорт, В.В. Чубаровский // Гигиена и санитария. — 2023. — Т. 102, №. 4. — С. 318–327.

25 Жеглова А.В. Оценка психоэмоционального состояния учителей общеобразовательной школы / А.В. Жеглова, И.В. Лапко, В.А. Кирьяков // Медицина труда и промышленная экология. — 2019. — № 9. — С. 628–628.

26 Сыманюк Э.Э. Особенности кризиса утраты профессии у педагогов позднего возраста / Э.Э. Сыманюк, Г.И. Борисов, А.А. Печеркина, В.В. Савельев // Образование и наука. — 2022. — Т. 24, №. 4. — С. 200–244.

27 Ковязина И.В. Взаимосвязь эмоционального выгорания и общего самочувствия личности у педагогов общеобразовательной школы / И.В. Ковязина, Е.В. Клименко, Г.В. Остякова // Профессиональное образование и рынок труда. — 2023. — № 1 (52). — С. 123–134.

28 Robinson L.E. Teachers, Stress, and the COVID-19 Pandemic: A Qualitative Analysis / L.E. Robinson, A. Valido, A. Drescher, et al. // School Mental Health. — 2023. — Vol. 15, No. 1. — P. 78–89.

29 Сыманюк Э.Э. Кризис утраты профессии учителя: анализ теоретических исследований / Э.Э. Сыманюк, А.А. Печеркина, Г.И. Борисов // Мир науки. Педагогика и психология. — 2020. — № 5. — С. 1–11.

30 Минасян Д.С. Влияние кортизола на когнитивные функции / Д.С. Минасян, М.А. Резникова, Д.В. Скалозуб // Международный журнал гуманитарных и естественных наук. — 2022. — № 12-2. — С. 46–49.

31 Maslach C. Understanding the Burnout Experience: Recent Research and Its Implications for Psychiatry / C. Maslach, M.P. Leiter // World Psychiatry. — 2016. — No. 15. — P. 103–111.

32 Хуснутдинова Р.Р. Психологические факторы эмоционального выгорания педагогов сельских школ Человеческий капитал / Р.Р. Хуснутдинова, Е.В. Коновалова. — 2022.

33 Цибульникова В.Е. Утомление педагогов в образовательном процессе как риск профессионального здоровья / В.Е. Цибульникова, Г.С. Кригер // Журнал «Школа Будущего». — 2016. — № 21. — С. 161–168.

34 Надеина Л.Е. Анализ профессиональной заболеваемости педагогов / Л.Е. Надеина // Вестник Донецкого педагогического института. — 2018. — № 2. — С. 134–140.

35 Чуракова А.В. Значение когнитивного компонента при формировании универсальных компетенций будущих педагогов / А.В. Чуракова // Вестник Сургутского государственного педагогического университета. — 2022. — № 4 (79). — С. 73–80.

36 Фомина Т.Г. Стресс в образовательной среде и его влияние на академическую успешность и психологическое благополучие обучающихся / Т.Г. Фомина, Е.В. Филиппова, А.В. Бурмистрова-Савенкова, В.И. Моросанова // Национальный психологический журнал. — 2024. — № 19(4). — С. 148–160.

А.Ә. Арыстанбай, Н.К. Смагулов, Г.М. Тыкежанова, М.В. Светлик

Жалпы білім беру мекемелері мұғалімдерінің бейімделуінің жас ерекшеліктері

Заманауи білім беру реформалары мұғалімдерден жоғары біліктілікті және жаңа жұмыс әдістеріне, соның ішінде цифрлық технологияларға тез бейімделуді талап етеді. Осы саладағы көптеген зерттеулерге қарамастан, мұғалімдердің кәсіби бейімделу мәселесі өзекті болып қала береді. Зерттеудің мақсаты: Жалпы білім беретін мектеп мұғалімдерінің кәсіби бейімделуіне әсер ететін факторларды анықтау, сондай-ақ педагогтардың бейімделуінің жас ерекшеліктерін және психофизиологиялық сипаттамаларын талдау. Зерттеуге Қарағандыдан жас ерекшеліктеріне қарай үш топқа бөлінген 137 мұғалім қатысты: 30 жасқа дейін, 30-45 жас және 45 жастан асқан. Мазасыздықты бағалау үшін Спилбергер-Ханин сынағы, әл-ауқатты, белсенділік пен көңіл-күйді субъективті бағалау үшін САН сынағы, психикалық өнімділікті зерттеу үшін Анфимовтың түзету кестелері, сондай-ақ физиологиялық әдістер (қан қысымын өлшеу, жүрек соғу жиілігінің өзгергіштігін талдау) қолданылды. Зерттеу барысында жалпы білім беретін мектеп мұғалімдері арасындағы реактивті мазасыздық деңгейі топтар арасында айтарлықтай айырмашылықтар болмағаны анықталды. Алайда әл-ауқатты, белсенділікті және көңіл-күйді талдауда жасына қарай әл-ауқаттың нашарлағанын, белсенділіктің шамалы ауытқулары болғанын және бірінші топта көңіл-күйдің жоғары екенін көрсетті. Ақыл-ой өнімділігі жасына қарай төмендеді, бұл қаралған және табылған белгілер санының азаюымен расталды. Сондай-ақ қан қысымының, жүрек соғу жиілігінің және реттеуші жүйелердің кернеу индексінің жоғарылауы байқалды. Симпатикалық жүйке орталығының белсенділігінің артуы және үлкен топтардағы реттеуші жүйелердің айқын функционалдық кернеуі байқалды. Зерттеу нәтижелері кәсіби қызметтің әртүрлі кезеңдерінде мұғалімдерді қолдау қажеттілігін растайды. Білім берудің цифрлық трансформациясы жағдайында стрестік жүктемені азайту және мұғалімдердің бейімделу деңгейін арттыру шаралары ерекше маңызды.

Кілт сөздер: жалпы білім беру мекемелерінің мұғалімдері, жас динамикасы, бейімделу, психофизиологиялық жағдайы, жұмысқа қабілеттілігі

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Возрастные особенности адаптации учителей общеобразовательных учреждений

Современные образовательные реформы требуют от учителей высокой квалификации и быстрой адаптации к новым методам работы, включая цифровые технологии. Несмотря на большое количество исследований в данной области, проблема профессиональной адаптации педагогов остается актуаль-

ной. Цель исследования: определение факторов, влияющих на профессиональную адаптацию учителей общеобразовательных школ, а также анализ возрастных особенностей адаптации и психофизиологических характеристик педагогов. В исследовании приняли участие 137 учителей из Караганды, разделенные на три возрастные группы: до 30 лет, 30–45 лет и более 45 лет. Использовались тест Спилбергера-Ханина для оценки тревожности, тест САН для субъективной оценки самочувствия, активности и настроения, корректурные таблицы Анфимова для изучения умственной работоспособности, а также физиологические методы (измерение артериального давления, анализ вариабельности сердечного ритма). В ходе исследования выявлено, что уровень реактивной тревожности среди учителей общеобразовательных школ не имел значительных различий между группами. Однако анализ самочувствия, активности и настроения показал, что самочувствие ухудшалось с возрастом, активность имела незначительные колебания, а настроение было выше в первой группе. Умственная работоспособность снижалась с возрастом, что подтверждалось уменьшением количества просмотренных и найденных знаков. Также наблюдался рост артериального давления, частоты сердечных сокращений и индекса напряжения регуляторных систем. Отмечено увеличение активности симпатического нервного центра и выраженное функциональное напряжение регуляторных систем в старших группах. Результаты исследования подтверждают необходимость поддержки педагогов на разных этапах профессиональной деятельности. Особенно важны меры по снижению стрессовой нагрузки и повышению уровня адаптации учителей в условиях цифровой трансформации образования.

Ключевые слова: учителя общеобразовательных учреждений, возрастная динамика, адаптации, психофизиологическое состояние, работоспособность

References

1 Kurkina, N.R., Potapova, L.N., & Imyarekov, S.M. (2024). Professionalnaia adaptatsiia molodykh pedagogov v usloviiakh tsifrovoi transformatsii obrazovaniia [Professional adaptation of young teachers in the context of digital transformation of education]. *Sovremennye problemy nauki i obrazovaniia — Modern Problems of Science and Education, 6* [in Russian].

2 Shavaleeva, G.M. (2022). Rol uchitelia v shkole i v obshchestve [The role of the teacher in school and in society]. *Otechestvennaia i zarubezhnaia pedagogika — Domestic and Foreign Pedagogy, 4,* 119–124 [in Russian].

3 Dadaeva, M.S., Magomadova, Z.S., & Minkailova, M.M. (2022). Professionalnaia adaptatsiia pedagogov kak kliuchevaia zadacha kadrovogo menedzhmenta obrazovatelnoi organizatsii [Professional adaptation of teachers as a key task of personnel management of an educational organization]. *Industrialnaia ekonomika — Industrial Economics, 3,* 240–243 [in Russian].

4 Kotova, S.A. (2010). Adaptatsiia v dolzhnosti i osvoenie professii uchitelia [Adaptation to the position and mastering the profession of a teacher]. *Narodnoe obrazovanie — People's Education, 8,* 121–127 [in Russian].

5 Sadovnikova, N.O. (2016). Professionalnyi krizis lichnosti pedagoga: soderzhanie i osnovnye priznaki [Professional personality crisis of a teacher: content and main features]. *Nauchnyi dialog — Scientific Dialogue,* 11(59), 400–411 [in Russian].

6 Mishkich, I.A., Baimakov, E.A., & Yushkova, O.I. (2021). Vliianie nervno-emotsionalnoi napriazhennosti trudovogo protsessa na organizm pedagogicheskikh i meditsinskikh rabotnikov [The influence of the nervous and emotional tension of the labor process on the body of pedagogical and medical workers]. *Meditsina truda i promyshlennaia ekologiia — Occupational Medicine and Industrial Ecology, 61*(4), 218–223 [in Russian].

7 Bomfim, M., Goulart, V., & de Oliveira, L. (2014). Teacher education in health: assessment, issues and tensions. *Comunicação saúde educação, 18,* 749–758.

8 Litchfield, P., Cooper, C., Hancock, Ch., & Watt, P. (2016). Work and Wellbeing in the 21st Century. *International Journal Environmental Research Public. Health, 13*(11), 1065.

9 Corrente, M., Ferguson, K., & Bourgeault, I. (2022). Mental Health Experiences of Teachers: A Scoping Review. *Journal of Teaching and Learning, 16*(1), 22–43.

10 Azarov, T.A., Mikerova, M.S., & Sokolov, N.A. (2024). Health in the system of teachers' values: health-saving behavior and lifestyle. *Public Health,* 4(4), 16–23.

11 Bagnetova, E.A., & Sharifullina, E.R. (2013). Professionalnye riski pedagogicheskoi sredy [Professional risks of the teaching environment]. *Fundamentalnye issledovaniia — Fundamental Research, 1,* 27–31 [in Russian].

12 Pecherkina, A., & Muslumov, R. (2018). Features of Professional Health of a Teacher at Different Stages of Professional Development. *The Fifth International Luria Memorial Congress "Lurian Approach in International Psychological Science"*, 700–710. Ekaterinburg [in Russian].

13 Kulganov, V. (2009). Psikhoemotsionalnoe napriazhenie i utomlenie uchitelia: mekhanizmy, diagnostika i profilaktika [Psycho-emotional stress and teacher fatigue: mechanisms, diagnosis and prevention]. *Izvestiia Rossiiskogo gosudarstvennogo pedagogicheskogo universiteta imeni A.I. Gertsena — Proceedings of the Russian State Pedagogical University named after A.I. Herzen, 100,* 131–142 [in Russian].

14 Shabas, S.G. (2023). Psikhofizicheskoe zdorove sovremennogo prepodavatelia kak uslovie kachestva obrazovatelnogo protsessa v rossiiskoi vysshei shkole [The psychophysical health of a modern teacher as a condition for the quality of the educational process in Russian higher education]. *Mir nauki. Pedagogika i psikhologiia — World of Science: Pedagogy and Psychology, 11*(4), 1–15 [in Russian].

15 Stepanov, E.G., Fasikov, R.M., & Didenko, N.A. (2010). Trud i zdorove uchitelei obshcheobrazovatelnykh shkol v sovremennykh usloviiakh [Work and health of secondary school teachers in modern conditions]. *Meditsina truda i promyshlennaia ekologiia — Occupational Medicine and Industrial Ecology, 6,* 45–49 [in Russian].

16 Yob, F., Ghani, A., Pek, L., et al. (2022). The Factors Affecting School Teachers' Wellbeing during Pandemic Era: A Scoping Review. *Open access, 11*(4), 887–904.

17 Pankov, V.A., Katamanova, E.V., & Slivnicyna, N.V. (2022). Usloviia truda i sostoianie zdorovia pedagogov obshcheobrazovatelnykh organizatsii (obzor literatury) [Working conditions and health status of teachers of educational institutions (literature review)]. *Gigiena i sanitariia — Hygiene and Sanitation, 101(8),* 940–946 [in Russian].

18 Chernikova, E.G. (2014). Osobennosti sotsialno-professionalnoi adaptatsii molodykh pedagogov obshcheobrazovatelnykh shkol [Features of social and professional adaptation of young teachers of secondary schools]. *Vestnik Cheliabinskogo gosudarstvennogo universiteta — Bulletin of Chelyabinsk State University, 3,* 4(333), 150–153 [in Russian].

19 Hanin, Yu.L. (1976). *Kratkoe rukovodstvo k shkale reaktivnoi i lichnostnoi trevozhnosti Ch.D. Spilbergera* [A Short Guide to the C.D. Spielberger Scale of Reactive and Personal Anxiety]. Leningrad [in Russian].

20 Doskin, V.A., Lavrenteva, H.A., Miroshnikov, M.P., & Sharai, V.B. (1973). Test differentsirovannoi samootsenki funktsionalnogo sostoianiia [The test of differentiated self-assessment of the functional state]. *Voprosy psikhologii — Questions of Psychology, 6,* 141 [in Russian].

21 Kuznetsov, A.P., Smelysheva, L.N., Sazhina, N.V., & Arhipova, O.A. (2019). Issledovanie umstvennoi rabotosposobnosti [Research on mental performance]. *Metodicheskie rekomendatsii k provedeniiu prakticheskikh i laboratornykh zaniatii dlia studentov napravlenii podgotovki — Methodological Recommendations for Conducting Practical and Laboratory Sessions for Students of Training Programs.* Kurgan. Izdatelstvo Kurganskogo gosudarstvennogo universiteta [in Russian].

22 Shlyk, N.I. (2022). *Variabelnost serdechnogo ritma u sportsmenov v trenirovochnom protsesse: metodicheskoe posobie* [Heart rate variability in athletes in the training process: methodical manual]. Izhevsk: Udmurtskii universitet [in Russian].

23 Baevskii, R.M., & Chernikova, A. (2017). Analiz variabelnosti serdechnogo ritma: fiziologicheskie osnovy i osnovnye metody provedeniia [Analysis of heart rate variability: the physiological basis and basic methods of conducting]. *Cardiometry, 10,* 68–80 [in Russian].

24 Kuchma, V.R., Sokolova, S.B., Rapoport, I.K., & Chubarovskii, V.V. (2023). Gigienicheskaia otsenka obraza zhizni, napriazhennosti i rezhima truda, sostoianiia zdorovia i psikhologicheskogo blagopoluchiia pedagogov [Hygienic assessment of the lifestyle, stress and work regime, health status and psychological well-being of teachers]. *Gigiena i sanitariia — Hygiene and Sanitation, 102*(4), 318–327 [in Russian].

25 Zheglova, A.V., Lapko, I.V., & Kiryakov, V.A. (2019). Otsenka psikhoemotsionalnogo sostoianiia uchitelei obshcheobrazovatelnoi shkoly [Assessment of the psycho-emotional state of secondary school teachers]. *Meditsina truda i promyshlennaia ekologiia — Occupational medicine and industrial ecology, 9,* 628–628 [in Russian].

26 Symanyuk, E.E., Borisov, G.I., Pecherkina, A.A., & Savelev, V.V. (2022). Osobennosti krizisa utraty professii u pedagogov pozdnego vozrasta [Features of the profession loss crisis among late-age teachers]. *Obrazovanie i nauka — Education and science, 24*(4), 200–244 [in Russian].

27 Kovyazina, I.V., Klimenko, E.V., & Ostyakova, G.V. (2023). Vzaimosviaz emotsionalnogo vygoraniia i obshchego samochuvstviia lichnosti u pedagogov obshcheobrazovatelnoi shkoly [The relationship between emotional burnout and the general well-being of the individual among secondary school teachers]. *Professionalnoe obrazovanie i rynok truda — Professional Education and the Labor Market, 1*(52), 123–134 [in Russian].

28 Robinson, L.E., Valido, A., Drescher A., et al. (2023). Teachers, Stress, and the COVID-19 Pandemic: A Qualitative Analysis. *School Mental Health, 15*(1), 78–89.

29 Symanyuk, E.E., Pecherkina, A.A., & Borisov, G.I. (2020). Krizis utraty professii uchitelia: analiz teoreticheskikh issledovanii [The crisis of the loss of the teaching profession: an analysis of theoretical research]. *Mir nauki. Pedagogika i psikhologiia — World of Science. Pedagogy and Psychology, 5,* 1–11 [in Russian].

30 Minasyan, D.S., Reznikova, M.A., & Skalozub, D.V. (2022). Vliianie kortizola na kognitivnye funktsii [The effect of cortisol on cognitive function]. *Mezhdunarodnyi zhurnal gumanitarnykh i estestvennykh nauk — International Journal of Humanities and Natural Sciences,* 12-2, 46–49 [in Russian].

31 Maslach, C., & Leiter, M.P. (2016). Understanding the Burnout Experience: Recent Research and Its Implications for Psychiatry. *World Psychiatry, 15,* 103–111.

32 Khusnutdinova, R.R., & Konovalova, E.V. (2022). *Psikhologicheskie faktory emotsionalnogo vygoraniia pedagogov selskikh shkol Chelovecheskii kapital* [Psychological factors of emotional burnout of rural school teacher's Human capital] [in Russian].

33 Cibulnikova, V.E., & Kriger, G.S. (2016). Utomlenie pedagogov v obrazovatelnom protsesse kak risk professionalnogo zdorovia [Teacher fatigue in the educational process as a risk to professional health]. *Zhurnal «Shkola Budushchego» — Journal "School of the Future", 21,* 161–168 [in Russian].

34 Nadeina, L.E. (2018). Analiz professionalnoi zabolevaemosti pedagogov [Analysis of professional morbidity of teachers]. *Vestnik Donetkogo pedagogicheskogo instituta — Bulletin of the Donetsk Pedagogical Institute,* 2, 134–140 [in Russian].

35 Churakova, A.V. (2022). Znachenie kognitivnogo komponenta pri formirovanii universalnykh kompetentsii budushchikh pedagogov [The importance of the cognitive component in the formation of universal competencies of future teachers]. *Vestnik Surgutskogo gosudarstvennogo pedagogicheskogo universiteta — Bulletin of the Surgut State Pedagogical University, 4*(79), 73–80 [in Russian].

36 Fomina, T.G., Filippova, E.V., Burmistrova-Savenkova, A.V., & Morosanova, V.I. (2024). Stress v obrazovatelnoi srede i ego vliianie na akademicheskuiu uspeshnost i psikhologicheskoe blagopoluchie obuchaiushchikhsia [Stress in the educational environment and its impact on academic success and psychological well-being of students]. *Natsionalnyi psikhologicheskii zhurnal — National Psychological Journal, 19*(4), 148–160 [in Russian].

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Optimization of *In vitro* Sterilization and Initial Cultivation Methods for Local Garlic (*Allium sativum* L.) Varieties

This article explores the optimization of sterilization and initial cultivation methods for local garlic (*Allium sativum* L.) varieties under *in vitro* conditions. Due to the biological characteristics of garlic, which rarely produces viable seeds, and the limited efficiency of conventional vegetative propagation methods, planting material is often susceptible to the accumulation of viral and fungal infections. Therefore, the use of biotechnological approaches represents an important direction for improving plant health and producing high-quality, uniform planting material. In this study, explants of the "Arman", "Zailiysky", and "Dungansky" varieties were cultured on Murashige and Skoog (MS) nutrient medium. Effective sterilization methods were selected, and optimal conditions for growth were determined. According to the obtained results, all studied varieties demonstrated good morphogenetic responses and showed a high potential for micropropagation *in vitro*. This method enables the establishment of an *in vitro* collection of local garlic varieties and can be applied in agricultural production for obtaining high-quality planting material. Furthermore, this technology ensures biological safety and facilitates the rapid propagation of virus- and fungus-resistant forms of garlic.

Keywords: garlic, *in vitro*, micropropagation, explant, sterilization, collection.

Introduction

Garlic (*Allium sativum* L.) is one of the oldest agricultural crops, first cultivated in the countries of Central Asia [1]. It is considered one of the most widespread and important species of the *Allium* genus used both as food and for medicinal purposes worldwide [2]. Garlic is mainly propagated vegetatively; however, due to the difficulty in inducing flowering, its genetic improvement through breeding is limited [3]. Additionally, garlic bulbs cannot be stored for more than 6–8 months, requiring annual replanting. This process can become economically inefficient due to pest and pathogen infestations [4]. Prolonged storage in open fields leads to the accumulation of viral infections, resulting in reduced yield or complete crop loss [5]. Viral diseases of garlic cause significant yield losses globally [6], and several virus species can be transmitted throughout the plant's entire life cycle during vegetative propagation [7].

The absence of seed formation in garlic limits breeding and sanitation methods to clonal selection and meristem culture [8]. In recent years, *in vitro* techniques have been widely used for the conservation of rare and valuable plant species [9]. Virus elimination using meristem (shoot tips) culture has proven effective for vegetatively propagated crops, including garlic [10]. Clones obtained in this way exhibit higher productivity and improved quality [11], and tissue culture methods hold great potential for increasing yield and producing virus-free planting material [12]. Moreover, this method is also effective in genetic improvement, breeding, and conservation research on garlic [13]. For large-scale production of garlic bulbs, the use of meristem or shoot tips for micropropagation is more efficient compared to other explant sources [14].

The relevance of this topic is associated with the growing demand in agriculture for high-quality, disease- and pest-free planting materials, as well as the need to limit the spread of viral and fungal diseases. Micropropagation technology under *in vitro* conditions allows for the elimination of various biotic threats through the cultivation of explants in an aseptic environment.

The aim of this study is to establish an *in vitro* collection of domestic garlic (*Allium sativum*) varieties preserved in the genetic fund of the "Kazakh Research Institute of Fruit and Vegetable Growing" LLP and to determine effective methods for sterilization and initial cultivation. The theoretical and practical significance of the research lies in optimizing sterilization techniques for garlic explants and improving the composition

of the nutrient medium using the *in vitro* method. By adapting the obtained plant samples to ex vitro conditions, it becomes possible to select disease-resistant and locally adapted forms, thereby expanding their potential use in agricultural production. Effective nutrient media and sterile conditions accelerate the microipropagaton of garlic and serve as a foundation for the development of seed production within the domestic biotechnology sector.

Micropropagation of garlic has been studied in many countries, and various combinations of nutrient media and growth regulators have been identified as effective. However, systematic studies on the *in vitro* cultivation and micropropagation of local garlic varieties have not previously been conducted in Kazakhstan. Therefore, the novelty of this study lies in the fact that, for the first time in Kazakhstan, the cultivation and adaptation characteristics of local garlic varieties under *in vitro* conditions have been investigated. The obtained results can serve as a basis for future selection of disease-resistant and high-yielding garlic forms**.**

The results of this research will contribute to the biotechnological modernization of garlic production in Kazakhstan, facilitate the production of high-quality planting material, and ensure stable agricultural productivity.

Experimental

The research was conducted in the biotechnology laboratory of the "Potato Breeding, Seed Production and Biotechnology" Department at the regional branch "Kainar" of the LLP "Kazakh Research Institute of Fruit and Vegetable Growing" (2024-2025).

As research objects, three domestic garlic (*Allium sativum*) varieties developed through the institute's breeding program were selected: "Arman", "Zailiyskiy", and "Dunganskiy".

Arman—a variety developed by the Kazakh Research Institute of Potato and Vegetable Growing, authored by Lakhin A.S. It is a mid-season, autumn-planted, bolting variety with a pungent taste, intended for general purposes. The bulbs are flat, white with a purple hue. On average, it has 10 large cloves weighing 6–9 g. It is disease-resistant with a yield of 6–10 t/ha. Realization is up 90 %, and the storage duration is 4-5 months. Since 1997, it has been recommended for cultivation in the Almaty region [15].

Zailiyskiy—a variety developed by Erenburg P.M. through mass selection from a local Dungan garlic population. It is a mid-season, autumn-planted, bolting variety. The bulbs are round-flat, white with a purple tint, weighing 35–55 g. It contains 5–8 cloves per bulb, each weighing 6–8 g. The average yield is 8–12 t/ha. It is resistant to cold and has a storage period of 4-5 months. Since 1955, it has been approved for cultivation in several regions [15].

Dunganskiy—a local Kazakh variety. It has a pungent flavor, is autumn-planted, and produces flower stalks. The growing season from sprouting to harvest is 120–130 days. The bulbs are round-flat with a purple tint and weigh 30–70 g. Each bulb has 5–9 cloves. The yield is 5.0–7.0 t/ha. Since 1959, it has been recommended for cultivation in the Zhambyl and South Kazakhstan regions [15].

The research was based on *in vitro* micropropagation methods. As explants, vegetative parts of garlic—specifically shoots derived from the root system—were used.

Sterilization procedures were carried out in several stages:

Pre-cleaning—Garlic samples were washed in a laundry soap solution and then rinsed 2-3 times in distilled water.

Chemical sterilization—Samples were treated in sodium hypochlorite solutions of varying concentrations for 2-3 minutes and then rinsed again 2-3 times in distilled water.

Aseptic processing—In a laminar flow cabinet, the outer scales of the garlic bulbs were removed, and shoots emerging from the root system were excised.

The explants were transferred into a pre-prepared nutrient medium. A modified Murashige and Skoog (MS) medium supplemented with phytohormones (0.5 mg/L indole-3-acetic acid(IAA) and 1.0 mg/Lbenzylaminopurine(BAP)) was used as the nutrient medium: MS salt base — 2.17 g; Sucrose — 15 g; Agar — 3.5 g; Thiamine HCl (Vitamin B1) — 0.5 mg/L; Pyridoxine HCl (Vitamin B6) — 0.25 mg/L; Total medium volume — 0.5 L.

After inoculation, the explants were placed in a phytotron room under controlled light conditions at a temperature of 25°C for cultivation.

Explants were cultivated in 50 mL glass test tubes, with one explant placed in each tube. As a nutrient medium, the basal Murashige and Skoog (MS) medium without the addition of phytohormones was used. The cultures were maintained under controlled phytotron conditions at 25 ± 2°C, with a 16-hour photoperiod

and 8 hours of darkness, and a light intensity of approximately 3000 lx. Each experimental variant included 30 explants and was conducted in three replications.

The obtained data were processed using Excel and Statistica 10.0 software. The results were presented as mean values ± standard error (SE), and the significance of differences was determined using Student's t-test and Duncan's multiple range test ($p < 0.05$).

Results and Discussion

During the study, three garlic cultivars—"Arman", "Zailiyskiy", and "Dunganskiy"—were cultivated under *in vitro* conditions. Effective sterilization methods were applied (Tab. 1), and all cultivars successfully adapted to the nutrient medium and began to grow without infection.

Table 1

Optimization of garlic explant sterilization methods

№	Garlic Variety	NaOCl Concentration, %	Treatment Time, minutes	Contamination Rate, %	Viability, %	Necrosis rate, %
1	"Arman"	0.5	3	40 ± 2.1*	50 ± 2.8	10 ± 1.5
		1.0	2	15 ± 1.2*	75 ± 3.0*	10 ± 1.3
		2.0	3	5 ± 0.8*	40 ± 2.5*	55 ± 3.2*
2	"Zailiyski"	0.5	3	35 ± 1.9*	55 ± 2.7*	10 ± 1.6
		1.0	2	10 ± 1.1*	80 ± 3.2*	10 ± 1.3
		2.0	3	8 ± 0.9	45 ± 2.1	47 ± 2.8*
3	"Dunganskiy"	0.5	3	38 ± 1.7*	48 ± 2.3	14 ± 1.9
		1.0	2	12 ± 1.0*	77 ± 3.1*	11 ± 1.4
		2.0	3	7 ± 0.8	43 ± 2.2	50 ± 2.7*

* – accuracy of differences in $p \leq 0.05$

As shown in Table 1, different concentrations of sodium hypochlorite (0.5 %, 1.0 %, and 2.0 %) and exposure times (2-3 min) were tested for garlic explant surface sterilization. The treatment with 1.0 % NaOCl for 2 minutes proved to be the most effective for all three varieties. Under these conditions, the contamination rate was low (10–15 %), the viability of explants was high (75–80 %), and the necrosis rate remained minimal (10–11 %). At a lower concentration (0.5 %), contamination levels increased (35–40 %) and viability decreased. Conversely, treatment with 2.0 % NaOCl reduced contamination but caused high toxicity, leading to a significant decrease in explant viability (40–45 %) and a marked increase in necrosis (up to 50 %). Therefore, sterilization with 1.0 % NaOCl for 2 minutes can be considered the optimal method for obtaining viable garlic explants under *in vitro* conditions.

Table 2

Growth performance of garlic varieties under *in vitro* conditions

№	Variety Name	Observation Time	Description	Shoot height, cm
Medium		**MS**		
1	"Arman"	On the 3 rd day	Aseptic, well adapted, initial sprouting observed	–
		On the 7 rd day	Active growth phase	1.4 ± 0.1
		On the 14 rd day	Root initiation and bud development observed	4.0 ± 0.2*
2	"Zailiyskiy"	On the 3 rd day	Explant adapted to the nutrient medium	–
		On the 7 rd day	Shoot formation and elongation started	1.5 ± 0.1
		On the 14 rd day	Active rooting and bud differentiation	5.0 ± 0.3*

Continuation of Table 2

№	Variety Name	Observation Time	Description	Shoot height (cm)
Medium		**MS**		
3	"Dunganskiy"	On the 3 rd day	Explant adapted to the medium	–
		On the 7 rd day	Moderate growth activity observed	1.3 ± 0.1
		On the 14 rd day	Roots formed, buds clearly visible	4.0 ± 0.2*

* – accuracy of differences in $p \leq 0.05$

During the study, the adaptation, growth, and rooting processes of garlic explants were monitored on the 3rd, 7th, and 14th days of cultivation (Fig. 1). In the first three days, all varieties showed successful adaptation to the aseptic medium, with visible signs of sprouting. By the 7th day, the "Arman" variety entered the active growth phase (1.4 cm), while "Zailiyskiy" demonstrated slightly higher elongation (1.5 cm). The "Dunganskiy" variety exhibited a slower growth rate (1.3 cm). By the 14th day, all varieties displayed evident root and bud formation. The "Zailiyskiy" variety showed the highest shoot height (5.0 cm), whereas both "Arman" and "Dunganskiy" reached 4.0 cm. These findings indicate that the MS nutrient medium has a positive influence on the morphogenesis of garlic explants under *in vitro* conditions. Among the studied varieties, "Zailiyskiy" exhibited the best adaptation, vigorous growth, and strong rooting potential.

One of the important factors affecting plant morphogenesis under *in vitro* conditions is the mineral composition of the nutrient medium. According to Kalinin F.L. et al. [16], numerous studies have proposed various nutrient media depending on their mineral content. However, many researchers utilize different modified variants of the Murashige and Skoog (MS) medium for garlic propagation. The efficiency of clonal micropropagation largely depends on the proper selection of the nutrient medium. For *in vitro* clonal propagation of plants, researchers commonly employ media such as Murashige and Skoog (MS), Linsmaier and Skoog, Gamborg and Eveleg, Phillips, Heller, White, and Gautheret [17, 18]. Nonetheless, based on the outcomes of multiple experiments, it has been established that modified versions of the MS medium—with varied compositions of minerals, vitamins, cytokinins, and auxins—produce optimal effects for plant propagation. Successful *in vitro* introduction of plants also depends on the type of sterilizing agents used. The composition of sterilizing agents is selected according to the characteristics of the explant. The initial stage of the experiment involves the removal of saprophytic microflora from the plant explants before their introduction into *in virto* conditions, followed by placing them on nutrient media for growth [19].

a) After 3 days b) After 7 days

Figure 1. Monitoring of explants

The nutrient medium and growth conditions significantly influence the regeneration, rooting, and development of healthy plant forms from explants. This method plays an important role in selection, conservation of genetic resources, and identification of pathogen-resistant forms. According to the results of the study, monitoring of the garlic explants introduced into the *in vitro* nutrient medium after 14 days showed the initiation of root formation and the development of shoots up to 4-5 cm, as illustrated in Figure 2.

Figure 2. Observation of garlic explants after 14 days in *in vitro* conditions

According to global research experience, micropropagation of plants under *in vitro* conditions is used as a rapid and efficient method to multiply unique forms of specific cultivars, hybrids, and limited amounts of initial plant material. Compared to traditional vegetative propagation, the advantages of micropropagation include the high multiplication rate of desired plants regardless of the season. This method is widely applied for the conservation of gene pools of rare, endangered, and agriculturally valuable varieties of seeds, fruits, vegetables, and ornamental plants [20].

Conclusion

As a result of the study, the initial stages of *in vitro* cultivation of local garlic (*Allium sativum*) varieties were developed—including effective explant sterilization and optimization of primary growth conditions. The use of 1.0 % sodium hypochlorite for 2 minutes ensured the lowest contamination rate and the highest explant viability. The Murashige and Skoog (MS) nutrient medium had a positive effect on the initial growth and morphogenesis of garlic explants. Among the studied varieties, the "Zailiyskiy" variety demonstrated the highest growth activity and viability.

These results form an important foundation for improving the initial stage of *in vitro* micropropagation. Future studies will focus on developing an efficient method for micropropagation based on the obtained sterile plant material, optimizing the growth and rooting stages, as well as selecting disease-resistant forms and establishing a collection.

This research will be carried out at the "Kainar" regional branch of the LLP "Kazakh Research Institute of Fruit and Vegetable Growing". The obtained results will contribute to the biotechnological development of garlic cultivation in Kazakhstan and the production of high-quality, disease-resistant planting material.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript: **Matai Z.M.** — conceptualization, investigation, writing draft; **Zhantasov S.K.** — data curation, analysis, methodology, data collection; **Ibragimova G.M.** — investigation, data analysis.

References

1 Ghaemizadeh F. Combination of stem-disc dome culture and thermotherapy to eliminate Allexiviruses and Onion yellow dwarf virus from garlic (*Allium sativum cv. Hamedan)* / F. Ghaemizadeh, F. Dashti, G. Khodakaramian, H. Sarikhani // Archives of Phytopathology and Plant Protection. — 2014. — Vol. 47(5). — P. 499–507. https://share.google/SJmYnslJbBDQmj9aw

2 Haider S. *In virto* plantlet regeneration of local garlic (*Allium sativum L.*) accessions of Bangladesh / S. Haider, M. Hossain, S. Rahman, S. Sultana, T. Quddus, M. Chakrabor et al. // British Biotechnology Journal. — 2015. — Vol. 8(1). — P. 1–12. https://share.google/9YdahI6qFhy8YAWGL

3 Поляков А.В. Получение посадочного материала озимого чеснока из воздушных бульбочек в Нечерноземной зоне Российской Федерации: методические рекомендации / А.В. Поляков, Т.В. Алексеев. — М., 2018. — 16 с. https://share.google/VOsN5uGyOL88kexWk

4 Suzan V.G. The influence of changing environmental factors on winter garlic varieties / V.G. Suzan, N.M. Nimatulaev, N.V. Litvinenko, I.V. Grekhova // International agricultural journal. — 2023. — No. 2. — P. 785–798. https://share.google/LE5Yz5ya1PEg9BSSR

5 Скурина В.В. Сравнительная оценка коллекционных сортов озимого чеснока по урожайности / В.В. Скурина, И.Г. Кохтенкова // Овощи России. — 2021. — № 3. — С. 60–67. https://share.google/IubRBHd8rXwjpijLd

6 Калинин Ф.Л. Технология микроклонального размножения растений / Ф.Л. Калинин, Г.П. Кушнир, В.В. Сарнацкая. — Киев: Наукова думка, 1992. — 120 с.

7 Mahajan R. *In vitro* and cryopreservation techniques for conservation of snow mountain garlic / R. Mahajan // Protocols for in virto cultures and secondary metabolite analysis of aromatic and medicinal plants. — 2016. — P. 335–346. — New York: Humana Press. https://share.google/AX6P6I93DQhQb50fn

8 Mishra R.K. Management of major diseases and insect pests of onion and garlic: A comprehensive review / R.K. Mishra, R.K. Jaiswal, D. Kumar, P.R. Saabale, A. Singh // Journal of Plant Breeding and Crop Science. — 2014. — Vol. 6(11). — P. 160–170. https://share.google/ATMWRTmnkNzUEAZBP

9 Parrano L. Characterization of viruses associated with garlic plants propagated from different reproductive tissues from Italy and other geographic regions / L. Parrano, M. Afunian, D. Pagliaccia, G. Douhan, G. Vidalakis // Phytopathologia Mediterranea. — 2012. — Vol. 51(3). — P. 549–565. https://share.google/vTp5cRELR9qdgn1Bw

10 Reed B.M. Improving *in virto* mineral nutrition for diverse pear germplasm / B.M. Reed, S. Wada, J. DeNoma, R.P. Niedz // In vitro Cellular & Developmental Biology — Plant. — 2013. — Vol. 49(3). — P. 343–355. https://share.google/ruQUUIUSfuWBPWSaH

11 Sarasan V. Conservation *in vitro* of threatened plants — Progress in the past decade / V. Sarasan, R. Cripps, M.M. Ramsay, C. Atherton, M. McMichen, G. Prendergast, J.K. Rowntree // In vitro Cellular and Developmental Biology — Plant. — 2006. — Vol. 42(3). — P. 206. https://share.google/MzzlvlqJTocershwG

12 Taskin H. Use of tissue culture techniques for producing virus-free plant in garlic and their identification through Real-Time PCR / H. Taskin, G. Baktemur, M. Kurul, S. Buyukalaca // The Scientific World Journal. — 2013. — P. 781282. https://share.google/KQYtqRTd2HtBfuZaO

13 Vieira R.L. Efficient elimination of virus complex from garlic (*Allium sativum* L.) by cryotherapy of shoot tips / R.L. Vieira, A.L. da Silva, G.R. Zaffari, D.A. Steinmacher, H.P. de Freitas Fraga, M.P. Guerra // Acta Physiologiae Plantarum. — 2015. — Vol. 37. — P. 1733. https://share.google/ED2zwPwviwGbAYNEH

14 Турдиев Т.Т. Оптимизация условий клонального микроразмножения тополя (*Populus* L.) / Т.Т. Турдиев, Н.А. Серадж, З.Р. Мухитдинова, С.Н. Фролов, И.Ю. Ковальчук // Ізденістер, нәтижелер — Исследования, результаты. — 2017. — № 3(75). — С. 122–128. https://share.google/koLnJCt4s0MGniaSl

15 Водянова О.С. Лук / О.С. Водянова. — Алматы: Қайнар, 2007. — 90 с.

16 Улимбашев А.М. Влияние массы зубков на продуктивность озимого чеснока / А.М. Улимбаева // Материалы международной научно-практической конференции профессорско-преподавательского состава Санкт-Петербургского государственного аграрного университета. — Санкт-Петербург, 2016. — С. 143–148. https://share.google/gsH0XQT9qWFfP1zw9

17 Елисеева Н.А. Сравнительная характеристика коллекционных образцов озимого чеснока в условиях Крыма / Н.А. Елисеева, Ю.Н. Костанчук // Таврический вестник аграрной науки. — 2023. — № 2 (34). — С. 52–60. https://share.google/cIU8R8Dd2ICg1787x

18 Романов В.С. Регенерация растений озимого чеснока *in vitro* после обработки колхицином / В.С. Романов, О.В. Романова, Л.И. Герасимова, Т.М. Середин // Аграрная наука. — 2021. — № 11. — С. 125–128. DOI: 10.32634/0869-8155-2020-343-11-125-128.

19 Чевычелова Н.В. Влияние условий возделывания на развитие растений яровой пшеницы /Н. В. Чувычелова, С. В. Жаркова // Вестник Алтайского государственного аграрного университета. — 2023. — № 11 (229). — С. 12–19. https://doi.org/10.53083/1996-4277-2023-229-11-12-19

20 Середин Т.М. Селекция озимого чеснока по качеству продукции / Т. М. Середин, А. Ф. Агафонов, Л. И. Герасимова, А. В. Солдатенко, Л. В. Кривенков. — Омск, 2020. — 115 с. https://share.google/fTl5LX6q0sfTbla7Q

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In vitro әдісі арқылы отандық сарымсақ (*Allium sativum L.*) сорттарын залалсыздандыру және бастапқы өсіру әдістерін оңтайландыру

Мақалада отандық сарымсақтың (*Allium sativum L.*) сорттарын *in vitro* жағдайында залалсыздандыру және бастапқы өсіру әдістерін оңтайландыру мәселесі қарастырылған. Сарымсақтың тұқым түзбейтін биологиялық ерекшелігі мен дәстүрлі көбейту тәсілдерінің шектеулі тиімділігі оны вирустық және саңырауқұлақты ауруларға бейім етеді. Сондықтан биотехнологиялық тәсілдерді қолдану өсімдіктерді сауықтыру мен сапалы, біркелкі отырғызу материалын алу үшін маңызды бағыт. Зерттеу барысында «Арман», «Заилийский» және «Дунганский» сорттарының эксплантты *Murashige & Skoog (MS)* қоректік ортасына енгізілді. Эксплантты залалсыздандырудың тиімді әдістері таңдалып, өсу процестеріне оңтайлы жағдайлар анықталды. Алынған нәтижелерге сәйкес, зерттелген сорттардың барлығы қоректік ортада жақсы морфогенез көрсетіп, микроклоналды көбейтуге бейімділік танытты. Бұл әдіс отандық сарымсақтың *in vitro* коллекциясын қалыптастыруға және ауыл шаруашылығында жоғары сапалы отырғызу материалын өндіруде қолдануға мүмкіндік береді. Сонымен қатар бұл технология өнімнің биологиялық қауіпсіздігін қамтамасыз етіп, вирустық және саңырауқұлақты ауруларға төзімді формаларды жедел көбейтуге жол ашады.

Кілт сөздер: сарымсақ, *in vitro*, микроклондау, эксплант, залалсыздандыру, коллекция

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Оптимизация методов стерилизации и первичного культивирования отечественных сортов чеснока (*Allium sativum L.*) методом *in vitro*

В данной статье рассматриваются вопросы оптимизации методов стерилизации и первичного культивирования отечественных сортов чеснока (*Allium sativum* L.) в условиях *in vitro*. Биологическая особенность чеснока — отсутствие семенного размножения, а также ограниченная эффективность традиционных методов вегетативного размножения — делает растение восприимчивым к вирусным и грибным заболеваниям. Поэтому применение биотехнологических подходов является важным направлением для оздоровления растений и получения качественного, однородного посадочного материала. В ходе исследования экспланты сортов «Арман», «Заилийский» и «Дунганский» были введены в питательную среду *Murashige & Skoog (MS)*. Были подобраны эффективные методы стерилизации эксплантов и определены оптимальные условия для их роста. Согласно полученным результатам, все исследованные сорта показали хорошую морфогенетическую активность и способность к микроклональному размножению в питательной среде. Разработанный метод позволяет создать *in vitro* коллекцию отечественных сортов чеснока и использовать её для производства высококачественного посадочного материала в сельском хозяйстве. Кроме того, данная технология обеспечивает биологическую безопасность продукции и открывает возможности для ускоренного размножения форм, устойчивых к вирусным и грибным заболеваниям.

Ключевые слова: чеснок, *in vitro*, микроклональное размножение, эксплант, стерилизация, коллекция

References

1 Ghaemizadeh, F., Dashti, F., Khodakaramian, G., & Sarikhani, H. (2014). Combination of stem-disc dome culture and thermotherapy to eliminate Allexiviruses and Onion yellow dwarf virus from garlic (*Allium sativum cv. Hamedan*). *Archives of Phytopathology and Plant Protection, 47*(5), 499–507. https://share.google/SJmYnslJbBDQmj9aw

2 Haider, S., Hossain, M., Rahman, S., Sultana, S., Quddus, T., Chakraborti, M., & et al. (2015). *In vitro* plantlet regeneration of local garlic (*Allium sativum* L.) accessions of Bangladesh. *British Biotechnology Journal, 8*(1), 1–12. https://share.google/9YdahI6qFhy8YAWGL

3 Polyakov, A.V., & Alekseeva, T.V. (2018). *Poluchenie posadochnogo materiala ozimogo chesnoka iz vozdushnykh bulbochek v Nechernozemnoi zone Rossiiskoi Federatsii: metodicheskie rekomendatsii* [Obtaining planting material of winter garlic

from aerial bulbils in the Non-Chernozem zone of the Russian Federation: methodological recommendations]. Moscow. https://share.google/VOsN5uGyOL88kexWk [in Russian].

4 Suzan, V.G., Nimatulaev, N.M., Litvinenko, N.V., & Grekhova, I.V. (2023). The influence of changing environmental factors on winter garlic varieties. *International agricultural journal*, 2, 785–798. https://share.google/LE5Yz5ya1PEg9BSSR

5 Skorina, V.V., & Kokhtenkova, I.G. (2021). Sravnitelnaia otsenka kollektsionnykh sortov ozimogo chesnoka po urozhainosti [Comparative assessment of collection varieties of winter garlic by yield]. *Ovoshchi Rossii — Vegetable crops of Russia*, 3, 60–67. https://share.google/IubRBHd8rXwjpijLd [in Russian].

6 Kalinin, F.L., Kushnir, G.P., & Sarnatskaya, V.V. (1992). *Tekhnologiia mikroklonalnogo razmnozheniia rastenii* [Technology of Micropropagation of Plants]. Kiev: Naukova Dumka https://share.google/bvd3ccfn81ixXcJUd [in Russian].

7 Mahajan, R. (2016). *In vitro* and cryopreservation techniques for conservation of snow mountain garlic. *Protocols for in vitro cultures and secondary metabolite analysis of aromatic and medicinal plants*, 335–346. New York: Humana Press https://share.google/AX6P6I93DQhQb50fn

8 Mishra, R.K., Jaiswal, R.K., Kumar, D., Saabale, P.R., & Singh, A. (2014). Management of major diseases and insect pests of onion and garlic: A comprehensive review. *Journal of Plant Breeding and Crop Science, 6*(11), 160–170. https://share.google/ATMWRTmnkNzUEAZBP

9 Parrano, L., Afunian, M., Pagliaccia, D., Douhan, G., & Vidalakis, G. (2012). Characterization of viruses associated with garlic plants propagated from different reproductive tissues from Italy and other geographic regions. *Phytopathologia Mediterranea, 51*(3), 549–565. https://share.google/vTp5cRELR9qdgn1Bw

10 Reed, B.M., Wada, S., DeNoma, J., & Niedz, R.P. (2013). Improving *in virto* mineral nutrition for diverse pear germplasm. *In vitro Cellular & Developmental Biology — Plant, 49*(3), 343–355. https://share.google/ruQUUIUSfuWBPWSaH

11 Sarasan, V., Cripps, R., Ramsay, M.M., Atherton, C., McMichen, M., Prendergast, G., & Rowntree, J.K. (2006). Conservation *in vitro* of threatened plants — Progress in the past decade. *In vitro Cellular and Developmental Biology — Plant, 42*(3), 206 https://share.google/MzzlvlqJTocershwG

12 Taskin, H., Baktemur, G., Kurul, M., & Buyukalaca, S. (2013). Use of tissue culture techniques for producing virus-free plant in garlic and their identification through Real-Time PCR. *The Scientific World Journal,* 781282. https://share.google/KQYtqRTd2HtBfuZaO

13 Vieira, R.L., da Silva, A.L., Zaffari, G.R., Steinmacher, D.A., de Freitas Fraga, H.P., & Guerra, M.P. (2015). Efficient elimination of virus complex from garlic (*Allium sativum* L.) by cryotherapy of shoot tips. *Acta Physiologiae Plantarum, 37*, 1733. https://share.google/ED2zwPwviwGbAYNEH

14 Turdiev, T.T., Serazh, N.A., Mukhitdinova, Z.R., Frolov, S.N., & Kovalchuk, I.Yu. (2017). Optimizaciia uslovii klonalnogo mikrorazmnozheniia topolia (*Populus* L.) [Optimization of clonal micropropagation conditions of poplar (*Populus L.*)]. *Izdenister, Nətizheler — Researches, Results, 3*(75), 122–128. https://share.google/koLnJCt4s0MGniaSl [in Russian].

15 Vodyanova, O.S. (2007). *Luk* [Onion]. Almaty: Qainar [in Russian].

16 Ulimbashev, A.M. (2016). Vliianie massy zubkov na produktivnost ozimogo chesnoka [Influence of clove mass on the productivity of winter garlic]. *Materialy Mezhdunarodnoi nauchno-prakticheskoi konferentsii professorsko-prepodavatelskogo sostava Sankt-Peterburgskogo gosudarstvennogo agrarnogo universiteta — Proceedings of the International Scientific and Practical Conference of the Faculty of Saint Petersburg State Agrarian University (pp. 143–148).* Saint Petersburg. https://share.google/gsH0XQT9qWFfP1zw9 [in Russian].

17 Eliseeva, N.A., & Kostanchuk, Yu.N. (2023). Sravnitelnaia kharakteristika kollektsionnykh obraztsov ozimogo chesnoka v usloviiakh Kryma [Comparative characteristics of collection samples of winter garlic in the conditions of Crimea]. *Tavricheskii vestnik agrarnoi nauki — Taurian Bulletin of Agrarian Science, 2*(34), 52–60. https://share.google/cIU8R8Dd2ICg1787x [in Russian].

18 Romanov, V.S., Romanova, O.V., Gerasimova, L.I., & Seredin, T.M. (2021). Regeneratsiia rastenii ozimogo chesnoka *in vitro* posle obrabotki kolkhitsinom [Regeneration of winter garlic plants *in vitro* after colchicine treatment]. *Agrarnaia nauka — Agrarian Science, 11, 125–128.* https://doi.org/10.32634/0869-8155-2020-343-11-125-128 [in Russian].

19 Chevychelova, N. V., & Zharkova, S.V. (2023). Vliianie uslovii vozdelyvaniia na razvitie rastenii yarovoi pshenitsy [The influence of cultivation conditions on the development of spring wheat plants]. *Vestnik Altaiskogo gosudarstvennogo agrarnogo universiteta — Bulletin of Altai State Agrarian University, 11*(229), 12–19. https://share.google/fTl5LX6q0sfTbla7Q [in Russian].

20 Seredin, T.M., Agafonov, A.F., Gerasimova, L.I., Soldatenko, A.V., & Krivenkov, L.V. (2020). *Selektsiia ozimogo chesnoka po kachestvu produktsii* [Breeding of winter garlic for product quality]. Omsk. https://share.google/fTl5LX6q0sfTbla7Q [in Russian].

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Identification of promising wheat lines resistant to tan spot (*Pyrenophora tritici-repentis*)

Wheat tan spot (yellow spot) is one of the most widespread and dangerous fungal diseases of wheat, caused by the phytopathogenic fungus *Pyrenophora tritici-repentis*. The aim of the study is to identify resistance to tan spot in promising wheat lines based on phenotypic indicators and molecular screening. A comprehensive assessment of wheat samples was carried out by the biomass index (NDVI), resistance to pathogens (PTR, AUDPC) and molecular markers. It was found that NDVI varies from 65 to 82, while high values (> 75) correlate with resistance (R^2 = 0.652), and low values (<70) — with susceptibility (S). Resistant samples (12 pcs., PTR = 5–10), including the Salamouni variety, and susceptible ones (7 samples, PTR = 15–35) were identified. As a result of molecular screening, PCR analysis of all studied genotypes showed the presence of the recessive gene tsn1 in 11 wheat genotypes (64.7 %). The integral indicator AUDPC (mean=70.8) demonstrated high variability (CV>50 %) and extreme values (up to 165). Statistical analysis confirmed the stability of NDVI (CV=6.5–6.7 %) and significant variability of PTR/AUDPC, reflecting mixed resistance. The results emphasize the need for an in-depth study of the relationship of molecular markers with resistance to tan spot.

Keywords: wheat, tan spot, resistance genes, molecular screening, resistance, NDVI, AUDPC.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple crops, with demand expected to increase to 330 kg per capita per year by 2050. Wheat production faces numerous threats, with an estimated 10–16 % of global wheat yields lost due to pests and diseases [1-2]. However, its productivity is significantly reduced by fungal pathogens such as brown rust (*Puccinia recondita*), yellow rust (P. *striiformis f. sp. tritici*), and tan spot (*Pyrenophora tritici-repentis*), which are among the most dangerous diseases capable of causing large-scale economic losses [3–8].

In Kazakhstan, control of leaf spot disease (tan spot) mainly relies on the use of resistant varieties and fungicide treatments. However, this approach, especially when chemical agents are applied untimely (before the appearance of critical symptoms), is often associated with high costs for farmers.

A key aspect of effective disease control is accurate diagnosis at early infection stages and monitoring of disease progression. Traditional methods, such as visual assessment of the affected tissue area, are subjective, labor-intensive, and require significant time.

A pressing issue for the Kazakh agricultural sector is the late detection of pathogens, which leads to substantial yield reductions due to insufficient disease spread control in the fields.

Tan spot is a relatively new wheat disease that is widespread in many countries. It was first detected in the 1940s in the USA and Canada, and during the 1980s–90s in Western European countries. In eastern Canada, tan spot and septoria occur together; the former in drier zones and the latter in more humid areas. Along with helminthosporium leaf blight, it is a widespread wheat disease in South Asian countries where wheat is grown in rice-wheat crop rotations. Its aggressiveness is promoted by cultivation of susceptible varieties and widespread adoption of zero-tillage technology. Tan spot is widespread on winter and spring wheat in the southern, southeastern, and northern regions of Kazakhstan. The first signs of the disease appeared during

stem elongation of winter wheat; at heading, leaf infection of the upper canopy was 25–50 %, increasing to 75–100 % at the milky ripeness stage, with premature leaf drying [9-10].

Three effector-dominant susceptibility gene interactions are known: ToxA-*Tsn1*, which causes necrotic symptoms, and ToxB-*Tsc2* and ToxC-*Tsc1*, both causing chlorosis. The *Tsn1*-ToxA interaction in leaf spot development depends on the host's genetic background, with the wheat gene *Tsn1* being the main factor determining susceptibility. Lamari et al. (2003) noted that this interaction follows a gene-for-gene inverse model. Genotypes lacking the *Tsn1* gene are insensitive to the toxin [11–14]. However, Adhikari et al. (2009) suggested that ToxA recognition via Tsn1 may activate key genes involved in the host's defense response and signaling pathways [14].

Integrated plant disease management requires combining multiple strategies for effective disease control. For tan spot, using resistant wheat varieties is the best option for sustainable disease management. Host resistance is also the most cost-effective and environmentally safe method to combat the disease. Therefore, breeding resistant wheat varieties should be a primary goal in managing tan spot, including assessing the susceptibility of embryonic tissue to the disease.

The aim of this study is to identify tan spot resistance in promising wheat lines based on disease phenotypes and molecular screening. The results will provide valuable knowledge to regional wheat breeders and phytopathologists involved in developing tan spot management strategies.

Experimental

Seventeen promising wheat lines grown in the Almaty region were used as research material. These wheat samples were tested for brown rust in laboratory studies at both the seedling stage and on mature plants under field conditions. The differential varieties Glenlea and Salamouni were used as controls for the negative and positive Tsn1 gene, which controls resistance to wheat yellow spot.

The studies were conducted during the 2024 growing season at the experimental site of the Kazakh Research Institute of Agriculture and Crop Production (KAZNIIzIR), Almalybak village, Almaty region (N43°14'210"; E076°41'282"). The experiment was designed as a completely randomized block design with three replications. All plots were surrounded by one-meter-wide strips planted with the highly susceptible Morocco variety. The size of each individual plot was 3 m² (3 m by 7 rows at 15 cm spacing). All recommended cultivation methods for commercial fields were applied, including fertilization, irrigation, and other management practices.

During the study period, weather conditions were favorable for the development of brown rust (http://weatherarchive.ru as of April 22, 2024). The amount of precipitation exceeded the norm, which increased environmental humidity and facilitated effective infection of plants with *Pyrenophora tritici-repentis* spores.

Phytopathological assessment of adult plants for yellow spot, including type and severity of infection, was recorded and evaluated on leaves in late May and early June, when the plots were at the maturation and milk-wax ripeness stages, respectively. For phytopathological evaluation of tan spot severity caused by *P. tritici-repentis*, the percentage of leaf area affected by yellow spot was assessed using the Saari and Prescott scale [15], originally developed for septoria and modified by O.Yu. Kremneva [16]. This leaf infection severity scale for wheat uses the following gradations: 0 % — very high resistance; 1–5 % — high resistance; 6–20 % — resistance; 21–30 % — susceptibility; 31–50 % — susceptibility; 51–80 % — high susceptibility; 81–100 % — very high susceptibility.

The area under disease progress curve (AUDP) was also assessed in the field, calculated using the formula by Wilcoxson et al. [17]:

$$S = 1/2S(x1+x2)\,(t1-t2) + \ldots.\,(xn-1+xn)\,(tn-tn-1)$$

where,

S — area under disease progress curve;

x1 — disease intensity at the first assessment, %;

x2 — disease intensity at the second assessment, %;

xn — disease intensity at the last assessment, %;

(t1-t2) — number of days between the first and second assessments;

(tn-tn-1) — number of days between the last and penultimate assessments.

Molecular Screening Methods for the Tsn1 Gene Conferring Wheat Resistance to Leaf Spot. Genomic DNA extraction was performed according to the method proposed by Riede et al. [18]. DNA was isolated

from 5-day-old wheat seedlings for each individual sample using the CTAB method. DNA concentration was measured spectrophotometrically at a wavelength of 260 nm. DNA concentration in the working PCR solution was adjusted to 20 ng/µl. The PCR reaction mixture (25 µl) contained 2.5 µl genomic DNA, 1 µl of each primer (1 pM/µl) (SigmaAldrich, USA), 2.5 µl dNTP mix (2.5 mM dCTP, dGTP, dTTP, and dATP) (ZAO "Sileks", Russia), 2.5 µl MgCl2 (25 mM), 0.2 µl Taq polymerase (5 units/µl) (ZAO "Sileks", Russia), 2.5 µl 10X PCR buffer, and 12.8 µl ddH2O. PCR amplification was carried out on a Mastercycler amplifier (Eppendorf, Germany). Amplification products were separated on a 2 % agarose gel in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8) with ethidium bromide. A 100-bp DNA ladder marker (Fermentas, Lithuania) was used to determine fragment sizes. Results were visualized using a gel documentation system (Gel Doc XR+, BIO-RAD, Hercules, USA) [18].

The Normalized Difference Vegetative Index (NDVI) was measured using a portable Green Seeker device (Trimble Navigation Limited, USA). NDVI ranges from 0.00 to 1.0; the higher the value, the greater the resistance to diseases.

Results and Discussion

Climatic conditions during the 2024 growing season at the experimental site of the Kazakh Research Institute of Agriculture and Crop Production (Almalybak village, Almaty region) were generally favorable for the development and progression of foliar fungal diseases, including tan spot caused by *Pyrenophora tritici-repentis*. According to meteorological data obtained from the regional weather archive (weatherarchive.ru), the study period was characterized by increased precipitation compared to the long-term average, particularly during the critical stages of wheat growth.

Excess rainfall contributed to elevated air and canopy humidity, creating optimal conditions for spore germination, infection, and subsequent disease spread. Moderate temperatures combined with frequent precipitation events enhanced leaf wetness duration, which is a key factor promoting successful penetration and colonization of host tissues by *P. tritici-repentis*. These environmental conditions facilitated effective natural infection pressure in the field, ensuring reliable differentiation of wheat genotypes based on their disease responses. Overall, the prevailing climatic conditions during the 2024 growing season provided a suitable background for the evaluation of tan spot severity and allowed for an accurate assessment of resistance levels under field conditions with high disease pressure.

A comprehensive assessment of 19 wheat accessions (including promising lines CP_1–CP_17 and control varieties Glenlea and Salamouni) was conducted across four key parameters: plant biomass index (NDVI), field resistance to pathogens (PTR, AUDPC), and the presence of the PTR resistance gene. The results revealed significant relationships for breeding. According to the research results, the NDVI biomass index, which reflects the photosynthetic activity of plants, ranged from 65 to 82 (Tab. 1). The highest values were demonstrated by: CP_4_2024 (80), CP_11_2024 (80), CP_12_2024 (82) — these accessions are distinguished by an optimal physiological state, which is typical of stress-resistant plants. The lowest NDVI values were recorded for the promising CP_15_2024 line (65), with low values associated with severe disease pressure or exposure to abiotic stressors (e.g., drought). A positive correlation (R2 = 0.652) was identified: high NDVI values (>75) were associated with resistance (R), while low NDVI values (<70) were associated with susceptibility (S).

Table 1

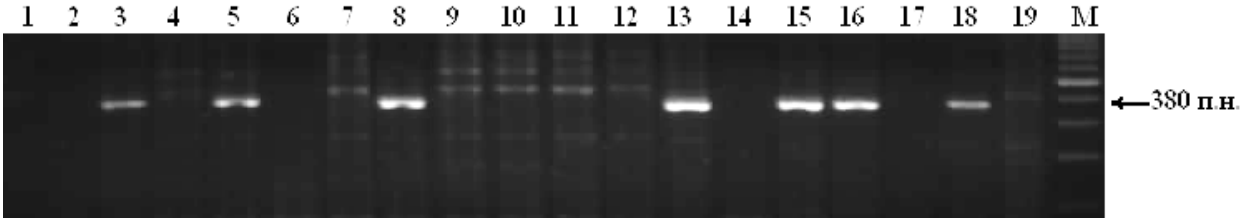
Results of a comprehensive study of promising wheat lines

№	Code of line	NDVI	Phytopathological evaluation to PTR	AUDPC	Molecular screening	
1	CP_1_2024	75	5	55	null	*tsn1*
2	CP_2_2024	78	10	65	null	*tsn1*
3	CP_3_2024	74	25	105	380	*Tsn1*
4	CP_4_2024	80	10	50	null	*tsn1*
5	CP_5_2024	70	25	110	380	*Tsn1*
6	CP_6_2024	72	10	60	null	*tsn1*
7	CP_7_2024	81	5	30	null	*tsn1*
8	CP_8_2024	70	15	70	380	*Tsn1*
9	CP_9_2024	73	10	35	null	*tsn1*

Continuation of Table 1

№	Code of line	NDVI	Phytopathological evaluation to PTR	AUDPC	Molecular screening	
10	CP_10_2024	75	10	40	null	*tsn1*
11	CP_11_2024	80	5	50	null	*tsn1*
12	CP_12_2024	82	5	55	null	*tsn1*
13	CP_13_2024	68	25	120	380	*Tsn1*
14	CP_14_2024	71	10	65	null	*tsn1*
15	CP_15_2024	65	25	135	380	*Tsn1*
16	CP_16_2024	68	25	70	380	*Tsn1*
17	CP_17_2024	75	10	40	null	*tsn1*
18	Glenlea	69	35	165	380	*Tsn1*
19	Salamouni	77	5	25	null	*tsn1*

Based on the phytopathological evaluation (PTR), resistant (R) accessions were identified: 12 accessions with damage scores of 5–10 (CP_1_2024, CP_2_2024, CP_4_2024, CP_6_2024, CP_7_2024, CP_9_2024, CP_10_2024, CP_11_2024, CP_12_2024, CP_14_2024, CP_17_2024 and the control cultivar Salamouni). Seven accessions with scores of 15–35 were classified as susceptible (S). The Glenlea cultivar had the highest score (35), confirming its status as a control cultivar for susceptibility. Molecular screening using PCR analysis of all studied genotypes revealed the presence of the recessive tsn1 gene in 11 wheat genotypes (64.7 %) and the presence of the dominant Tsn1 gene, which is resistant to the PTR ToxA toxin. Six samples (35.3 %) were found to contain DNA fragments of 380 bp (Fig. 1). Of the 19 wheat samples studied, six samples were found to contain 380 bp, and these genotypes are sensitive to the PtrToxA toxin.



1-CP_1_2024, 2- CP_2_2024, 3- CP_3_2024, 4- CP_4_2024, 5- CP_5_2024, 6- CP_6_2024, 7- CP_7_2024, 8- CP_8_2024, 9- CP_9_2024, 10- CP_10_2024, 11- CP_11_2024, 12- CP_12_2024, 13- CP_13_2024, 14- CP_14_2024, 15- CP_15_2024, 16- CP_16_2024, 17- CP_17_2024, 18- Glenlea (sensitive to toxin PtrToxA), 19- Salamouni (insensitive to the toxin PtrToxA)

Figure 1. Products of wheat DNA amplification using primers to the *Xfcp623* locus associated with the *Tsn1/tsn1* resistance gene

Table 2 presents the complete descriptive statistics of the studied promising wheat lines. Descriptive statistical analyses were performed for four key traits: normalized difference vegetation index (NDVI), tan spot severity (PTR), area under the disease progress curve (AUDPC), and molecular screening results. A total of 19 observations were included for each variable, with no missing values, ensuring complete datasets for all statistical evaluations.

NDVI values ranged from 65.0 to 82.0, with a mean of 73.84 and a median of 74.0, indicating relatively high and stable canopy greenness across the tested genotypes. The low coefficient of variation (CV = 6.5 %) and narrow interquartile range reflected limited dispersion and a high degree of uniformity among genotypes. The distribution of NDVI values was approximately symmetrical, as indicated by near-zero skewness (Pearson = 0.07) and negative kurtosis (Pearson = −1.02), suggesting a slightly flattened distribution relative to normality.

Tan spot severity (PTR) showed a much wider range, varying from 5.0 to 35.0, with a mean of 14.21 and a median of 10.0. The relatively high coefficient of variation (CV = 63.8 %) reflected substantial variability in disease response among genotypes. Positive skewness (Pearson = 0.76) indicated a right-tailed dis-

tribution, with a greater frequency of genotypes exhibiting low to moderate disease severity and fewer highly susceptible entries.

AUDPC values ranged from 25.0 to 165.0, with a mean of 70.79 and a median of 60.0, confirming pronounced differences in disease progression over time. The coefficient of variation (CV = 52.9 %) indicated considerable heterogeneity in resistance levels. The distribution was positively skewed (Pearson skewness = 1.03), suggesting that most genotypes exhibited relatively low AUDPC values, while a limited number showed strong disease development. The slightly positive kurtosis (Pearson = 0.13) reflected moderate peakedness of the distribution.

Molecular screening data were binary (0/1) and ranged from 0 to 1, with a mean value of 0.37, indicating that 36.8 % of the evaluated genotypes carried the targeted molecular marker(s). The distribution was characterized by a high coefficient of variation (CV = 130.9 %), which is typical for binary traits. The median value of 0.0 confirmed that the majority of genotypes lacked the marker, while positive skewness reflected the lower frequency of marker-positive entries.

Table 2

Results of descriptive statistics for the studied promising wheat lines

Statistic	NDVI	PTR	AUDPC	Molecular screening
Nbr. of observations	19	19	19	19
Nbr. of missing values	0	0	0	0
Obs. without missing data	19	19	19	19
Sum of weights	19	19	19	19
Breakdown per subsample (%)	100,000	100,000	100,000	100,000
Minimum	65,000	5,000	25,000	0,000
Maximum	82,000	35,000	165,000	1,000
Freq. of minimum	1	5	1	12
Freq. of maximum	1	1	1	7
Range	17,000	30,000	140,000	1,000
1st Quartile	70,000	7,500	45,000	0,000
Median	74,000	10,000	60,000	0,000
3rd Quartile	77,500	25,000	87,500	1,000
Sum	1403,000	270,000	1345,000	7,000
Mean	73,842	14,211	70,789	0,368
Variance (n)	22,975	82,271	1400,693	0,233
Variance (n-1)	24,251	86,842	1478,509	0,246
Standard deviation (n)	4,793	9,070	37,426	0,482
Standard deviation (n-1)	4,925	9,319	38,451	0,496
Variation coefficient (n)	0,065	0,638	0,529	1,309
Variation coefficient (n-1)	0,067	0,656	0,543	1,345
Skewness (Pearson)	0,066	0,764	1,029	0,546
Skewness (Fisher)	0,072	0,831	1,119	0,593
Skewness (Bowley)	-0,067	0,714	0,294	1,000
Kurtosis (Pearson)	-1,022	-0,724	0,125	-1,702
Kurtosis (Fisher)	-0,955	-0,561	0,562	-1,856
Standard error of the mean	1,130	2,138	8,821	0,114
Lower boundon mean (95 %)	71,469	9,719	52,257	0,130
Upper boundon mean (95 %)	76,216	18,702	89,322	0,607
Standard error of the variance	8,084	28,947	492,836	0,082
Lower boundon variance (95 %)	13,846	49,583	844,155	0,140
Upper boundon variance (95 %)	53,036	189,917	3233,383	0,537

Continuation of Table 2

Statistic	NDVI	PTR	AUDPC	Molecular screening
Mean absolute deviation	4,061	7,950	29,584	0,465
Median absolute deviation	4,000	5,000	20,000	0,000
Geometric mean	73,686	11,572	62,228	-
Geometric standard deviation	1,069	1,942	1,678	-
Harmonic mean	73,531	9,523	55,122	-
nIQR	5,560	12,973	31,505	0,741
Qn	6,178	10,297	30,890	0,000

For all traits, 95 % confidence intervals for the mean were calculated, providing reliable estimates of central tendency under field conditions. Overall, the statistical analysis demonstrated low variability for NDVI, moderate to high variability for disease-related traits (PTR and AUDPC), and high heterogeneity in molecular screening results, allowing robust differentiation of wheat genotypes based on physiological performance, disease resistance, and genetic background.

Figure 2 presents Box plot visualizations of the studied parameters in the promising wheat lines. The obtained data emphasize the need for deeper investigation of the relationships between molecular markers and disease resistance.

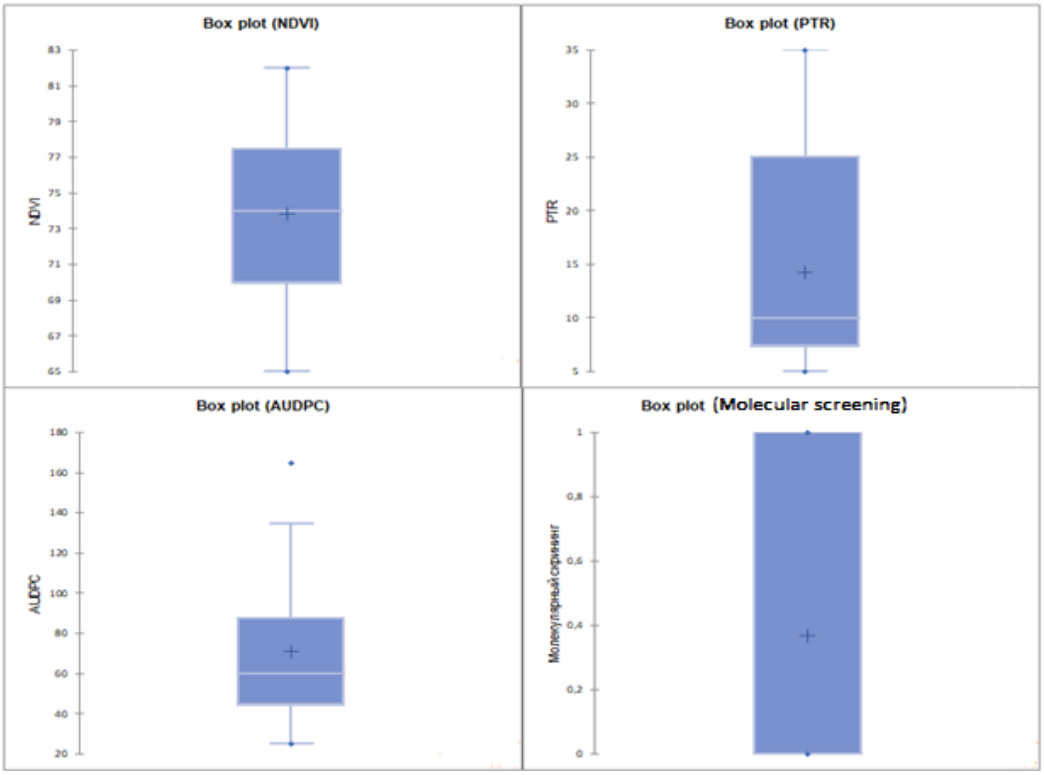


Figure 2. Box plot diagram of the studied promising wheat lines

Box plot visualizations illustrating the distribution of NDVI, tan spot severity (PTR), AUDPC, and molecular screening results among the studied promising wheat lines. The box plots display the median, interquartile range, and minimum–maximum values for each trait, highlighting differences in variability and distribution patterns. NDVI values show a relatively narrow dispersion, indicating stable physiological performance across most genotypes, whereas PTR and AUDPC exhibit wider variability, reflecting contrasting levels of disease response and progression. Molecular screening data demonstrate a binary distribution corresponding to the presence or absence of the *Tsn1/tsn1* alleles. Overall, the box plot analysis emphasizes heterogeneity among wheat genotypes and underscores the importance of integrating phenotypic and molecular data to better understand the genetic basis of resistance to *Pyrenophora tritici-repentis*.

Conclusion

The results of this study confirm that the climatic conditions of the 2024 growing season provided a reliable and informative background for evaluating wheat resistance to tan spot caused by *Pyrenophora tritici-repentis*. Elevated precipitation and moderate temperatures ensured high natural infection pressure, allowing clear differentiation of genotypes based on physiological performance, disease severity, and genetic composition. Integrated analysis of NDVI, phytopathological traits (PTR and AUDPC), and molecular screening revealed substantial variability among the 19 studied wheat accessions. NDVI values showed low variability and strong association with disease response, confirming NDVI as a reliable indicator of plant health under disease pressure. A positive relationship between NDVI and resistance ($R^2 = 0.652$) demonstrated that genotypes with higher biomass stability exhibited reduced disease severity and slower disease progression. Phytopathological assessments identified twelve resistant accessions with low PTR and AUDPC values, while seven accessions were classified as susceptible. The cultivar Glenlea consistently exhibited the highest disease severity, validating its role as a susceptible control, whereas Salamouni confirmed its resistance under field conditions. Molecular screening revealed that 35.3 % of genotypes carried the dominant *Tsn1* allele associated with sensitivity to PtrToxA, while 64.7 % carried the recessive *tsn1* allele. In general, the presence of *tsn1* corresponded to reduced disease severity; however, inconsistencies between molecular and phenotypic data highlight the quantitative nature of tan spot resistance and the involvement of additional resistance loci. Overall, the combined use of NDVI-based phenotyping, disease progression metrics, and molecular markers enabled robust identification of promising wheat lines with enhanced resistance to tan spot. These genotypes represent valuable breeding material for developing cultivars with improved and durable resistance under the agro-climatic conditions of southeastern Kazakhstan.

Conflict of interest

The authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Raimbekova B.T.** — Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing original draft; editing; **Anuarova L.E.** — data curation, investigation, formal analysis, methodology; **Kyrbasova E.A.** — resources, software, supervision, visualization; **Imanova E.M**. — resources, validation, visualization; **Sartayeva A.A.** — supervision, validation, original draft writing; **Doszhanova A.S**. — investigation, data curation.

References

1 Food and Agriculture Organization of the United Nations (2020). *World Food Situation — FAO Cereal Supply and Demand Brief*. Rome, Italy.

2 United States Department of Agriculture (USDA) (2020). *World Agricultural Production. Circular Series WAP 9–20. Foreign Agricultural Service, Gobal Market Analysis*. Washington, DC, USA.

3 Kolmer, J.A. (2005). Tracking wheat rust on a continental scale. *Current Opinion Plant Biology, 8,* 441–449.

4 Hovmøller, M.S., Walter, S., & Justesen, A.F. (2010). Escalating threat of wheat rusts. *Science, 329*, 369.

5 El Jarroudi, M., Kouadio, L., Giraud, F., Delfosse, P., & Tychon, B. (2014). Brown rust disease control in winter wheat: II. Exploring the optimization of fungicide sprays through a decision support system. *Environmental Science Pollution Research, 21*, 4809–4818.

6 Beddow, J.M., Pardey, P.G., Chai, Y., Hurley, T.M., Kriticos, D.J., Braun, H. -J., Park, R.F., Cuddy, W.S., & Yonow, T. (2015). Research investment implications of shifts in the global geography of wheat stripe rust. *Nat. Plants, 1*, 15132.

7 El Jarroudi, M., Kouadio, L., Beyer, M., Junk, J., Ho Mann, L., Tychon, B., Maraite, H., Bock, C.H., & Delfosse, P. (2015). Economics of a decision–support system for managing the main fungal diseases of winter wheat in the Grand-Duchy of Luxembourg. *Field Crops Res., 172*, 32–41.

8 Ali, S., Rodriguez-Algaba, J., Thach, T., Sørensen, C.K., Hansen, J.G., Lassen, P., Nazari, K., Hodson, D.P., Justesen, A.F., & Hovmøller, M.S. (2017). Yellow rust epidemics worldwide were caused by pathogen races from divergent genetic lineages. *Front. Plant Sci., 8*, 1057.

9 Faris, J.D., Liu, Z., & Xu, S.S. (2013). Genetics of tan spot resistance in wheat. *Theoretical and Applied Genetics, 126*, 2197–2217. https://doi.org/10.1007/s00122-013- 2157-y

10 Mofat, C.S., See, P.T., & Oliver, R.P. (2014). Generation of a ToxA knockout strain of the wheat tan spot pathogen *Pyrenophora tritici-repentis*. *Molecular Plant Pathology, 15,* 918–926. https://doi.org/10.1111/mpp.12154

11 Lamari, L., Strelkov, S.E., Yahyaoui, A., Orabi, J., & Smith, R.B. (2003). The identification of two new races of *Pyrenophora tritici-repentis* from the host center of diversity confirms a one-to-one relationship in tan spot of wheat. *Phytopathology, 93,* 391–396. https:// doi.org/10.1094/PHYTO.2003.93.4.391

12 Anderson, J.A., Efertz, R.J., Faris, J.D., Francl, L.J., & Meinhardt, S.W. (1999). Genetic analysis of sensitivity to *Pyrenophora tritici-repentis* necrosis-inducing toxin in durum and common wheat. *Phytopathology, 89*, 293–297. https://doi.org/10.1094/PHYTO.1999.89.4.293

13 Faris, J.D., Anderson, J.A., Francl, L.J., & Jordahl, J.G. (1996). Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. *Phytopathology, 86,* 459–463. https://doi.org/10.1094/Phyto-86-459

14 Gamba, F.M., Bassi, F.M., & Finckh, M.R. (2017). Race structure of *Pyrenophora tritici-repentis* in Morocco. *Phytopathologia Mediterranea, 56,* 119–126. https://doi.org/10.14601/Phytopathol_Mediterr-18830

15 Saari, E. E., & Prescott, L. M. (1975). A scale for appraising the foliar intensity of wheat diseases. *Plant Dis. Rep., 59,* 377–380.

16 Kremneva, O. Yu., & Volkova, G. V. (2007). *Diagnostics and methods for assessing wheat resistance to the causative agent of yellow leaf spot. Methodological recommendations*. Moscow.

17 Wilcoxson, R. D., Atif, A. H., & Skowmand, B. (1974). Slow rusting of wheat varieties in the field correlated with stem rust severity on detached leaves in the greenhouse. *Plant disease reporter, 58*(12), 1085–1087.

18 Riede, C.R., & Anderson, J.A. (1996). Linkage of RFLP markers to an aluminum tolerance gene in wheat. *Crop Science, 36*, 905–909.

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Пиренофорозға төзімді (*Pyrenophora tritici-repentis*) перспективті бидай линияларын сәйкестендіру

Бидай пиренофорозы (сары дақ) *Pyrenophora tritici-repentis* фитопатогенді саңырауқұлағы қоздыратын кең таралған және қауіпті ауруларының бірі. Зерттеудің мақсаты — фенотиптік көрсеткіштер мен молекулалық скрининг негізінде перспективті бидай линияларының пиренофорозға төзімділігін анықтау. Бидай үлгілерін кешенді бағалау биомасса индексі (NDVI), патогенге төзімділік (PTR, AUDPC) және молекулалық маркерлер негізінде жүргізілді. NDVI 65-тен 82-ге дейін өзгеретіні анықталды, яғни жоғары мәндер (>75) қарсылықпен (R^2=0,652), ал төмен мәндер (<70) — сезімталдықпен (S). Төзімді үлгілер (12 дана, PTR=5–10), оның ішінде Salamouni сорты және төзімсіз үлгілер (7 үлгі, PTR=15–35) айқындалды. Молекулярлық скрининг нәтижесінде барлық зерттелген генотиптердің ПТР талдауы бидайдың 11 генотипінде (64,7 %) рецессивті *tsn1* генінің болуын көрсетті. Интегралды AUDPC индексі (орташа = 70,8) жоғары өзгергіштік (CV>50 %) және экстремалды мәндерді (165-ке дейін) көрсетті. Статистикалық талдау NDVI тұрақтылығын (CV=6,5–6,7 %) және аралас қарсылықты көрсететін PTR/AUDPC айтарлықтай өзгермелілігін растады. Нәтижелер тотығу дақтарына төзімділікпен молекулалық маркерлердің байланысын одан әрі зерттеу қажеттілігін көрсетеді.

Кілт сөздер: бидай, пиренофороз, төзімділік гендер, молекулалық скрининг, төзімділік, NDVI, AUDPC

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Идентификация перспективных линий пшеницы устойчивых к пиренофорозу (*Pyrenophora tritici-repentis*)

Пиренофороз пшеницы (желтая пятнистость) — одно из наиболее распространённых и опасных грибковых заболеваний пшеницы, вызываемое фитопатогенным грибом *Pyrenophora tritici-repentis.* Целью исследования является идентификация устойчивости к пиренофорозу перспективных линий пшеницы на основе фенотипических показателей и молекулярного скрининга. Проведена комплексная оценка образцов пшеницы по индексу биомассы (NDVI), устойчивости к патогенам (PTR, AUDPC) и молекулярным маркерам. Установлено, что NDVI варьирует от 65 до 82, при этом высокие значения (>75)

коррелируют с устойчивостью (R^2=0,652), а низкие (<70) — с восприимчивостью (S). Выделены устойчивые образцы (12 шт., PTR=5–10), включая сорт Salamouni, и восприимчивые (7 образцов, PTR=15–35). В результате молекулярного скрининга ПЦР-анализ всех исследованных генотипов показал наличие рецессивного гена *tsn1* у 11 генотипов пшеницы (64,7 %). Интегральный показатель AUDPC (среднее=70,8) продемонстрировал высокую вариабельность (CV>50 %) и экстремальные значения (до 165). Статистический анализ подтвердил стабильность NDVI (CV=6,5–6,7 %) и значительную изменчивость PTR/AUDPC, отражающую смешанную устойчивость. Результаты подчеркивают необходимость углубленного изучения связи молекулярных маркеров с устойчивостью к пиренофорозу.

Ключевые слова: пшеница, пиренофороз, гены устойчивости, молекулярный скрининг, устойчивость, NDVI, AUDPC

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Review

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Anther culture in rice: from an experimental model to breeding practice

The anther culture method has become an effective tool in modern rice breeding, enabling a significant reduction in the breeding cycle through the rapid production of haploid and doubled haploid plants. Extensive global experience, particularly in major rice-producing countries such as China, Japan, and India, confirms the importance of androgenesis technology for developing new high-yielding and high-quality rice varieties. The efficiency of this method depends on multiple interacting factors, the most critical being genotype. Studies have shown that the japonica subspecies of rice is markedly more responsive and suitable for anther culture, whereas the indica subspecies is typically exhibits low callus induction and regeneration capacity. To overcome these limitations, inter-subspecific hybridization and optimization of culture protocols are commonly employed. This approach has also been successfully implemented in Kazakhstan, where several new rice lines and varieties have been developed. In particular, the cultivar Fatima demonstrates increased productivity (~5.1 t/ha) and valuable agronomic traits and has already been released for cultivation in the country's major rice-growing regions. Moreover, anther culture has enabled Kazakhstani breeders to obtain doubled haploid lines surpassing their parental forms in several agronomic characteristics, such as the glutinous variety Violetta. In conclusion, anther culture in rice holds considerable potential for accelerating the development of homozygous lines and novel genotypes. The integration of this approach with molecular tools such as marker-assisted selection (MAS) and CRISPR/Cas9 genome editing offers new opportunities for targeted gene combination and for expediting the development of improved rice varieties with desired traits.

Keywords: rice, male gametophyte, anther and microspore culture, androgenesis in vitro, biological, chemical, physical factors.

Introduction

One of the most significant areas of haploid plant biotechnology is anther culture in rice (Oryza sativa L.), which enables the production of homozygous lines in a single generation and significantly shortens the breeding cycle [1]. This technology is based on the phenomenon of androgenesis, that is, the reprogramming of microspores from the gametophyte to the sporophyte pathway [2-3]. This in turn leads to the emergence of regenerated haploid plants, which can subsequently be doubled to produce a diploid plant [4]. The anther culture method allows for the production of stable inbred lines without long-term pollination, making it a powerful tool for both applied breeding, which includes accelerated variety development, and for fundamental genetic research, such as QTL analysis, genomic mapping, and transgenesis [5-6]. Haploid rice varieties were first obtained in 1931 by Morinaga and Fukushima by crossing two varieties (Dekiyama ♀ x Bunketu ♂) [7]. In 1933, Nakamura [8] described the haploid rice plant, and the first successful experiments on androgenesis *in vitro* were conducted by Niizeki and Oono in 1968 [9] using anther cultures of six rice varieties. In 1970, Guha and co-authors identified the use of totipotent male cells in gametophytic rice plants [10]. In 1975, as a result of comparative experiments on nitrogen sources, the effective Chu medium for culturing haploids was developed, which subsequently influenced the creation of the now widely used N6 medium [11-12]. Since then, this method has undergone intensive development, and by the 1980s, the first androgenic rice varieties (Huayu I, Huayu II, Xin Xiu, Late Keng 959, Tunghua 1, Tunghua 2, Tunghua 3)

were obtained in China [13]. These varieties combined several beneficial properties, such as productivity, stability, and high grain quality. Today, the anther culture method is recognized worldwide as a necessary component of breeding program [14-15].

Despite its widespread use and effectiveness in breeding Japonica rice, it still faces limiting factors associated with Indica rice, including early anther necrosis, poor callus induction and proliferation, low green plant regeneration, and a high level of albinism in regenerated plants [16]. These issues stem from the molecular and physiological characteristics of microspores, which require comprehensive optimization, including selection of donor genotypes, precise selection of nutrient medium composition, cultivation conditions, and stress management [17-18]. Rice cultivation in Kazakhstan is export-oriented, making accelerated breeding and the development of resilient, high-quality rice varieties extremely pressing [19]. This article aims to comprehensively analyze the physical, chemical, and biological factors that influence the success of androgenesis, identify the weak points of the method, and discuss current, modern strategies for integration into molecular and genomic rice breeding in the country.

Experimental

A review of available sources was conducted using the Scopus/Web of Science database for the period 1930–2025. The following keywords were used in the search for literature sources: rice; physical, chemical, and biological factors of androgenesis; methods for rice androgenesis; strategies for integration; molecular and genomic rice breeding.

Review

Factors affecting the efficiency of in vitro androgenesis—biological, chemical, and physical conditions determining the success of anther culture

A review of the literature and practical experiments revealed that the success of rice anther and microspore culture in vitro is determined by the combined effects of three main groups of factors: biological, chemical, and physical. The interaction of these factors determines the ability of microspores to develop sporophytically, forming callus tissue and viable regenerants.

Biological Factors

Biological factors include the genotype, physiological state of the donor plant, and the developmental stage of the male gametophyte [3, 17, 18, 20]. The genotype of the donor plant, in turn, determines the embryogenic potential of rice microspores [21]. Japonica subspecies varieties are generally more responsive to androgenesis than indica varieties. These differences are due to plastid and nuclear genes responsible for chloroplast biogenesis and morphogenesis [21]. According to research results, anthers from fully developed and healthy plants have a higher embryogenesis potential, especially if optimal cultivation conditions were selected. A critical factor influencing the efficiency of *in vitro* androgenesis in anther culture is the quality of donor plants [1, 18, 20, 22]. The process of accelerated dihaploid production directly depends on the health of the plant, its shoots and ears. Two cultivation methods are most frequently used: controlled conditions in a greenhouse or phytotron chamber, or favorable field conditions in a nursery [23]. Based on experimental data, it has been shown that donor plants grown in field conditions outperform those obtained under controlled conditions [24]. Such regenerants develop significantly more shoots and have a developed ear with large anthers and microspores [24-25]. This difference is directly related to the number of viable microspores developing in the anthers and the nutritional status of the anther tissues [26]. Thus, the stage of microspore development is a critical factor that influences the direction of cell differentiation [27]. It has been shown that microspores at the middle or late mononuclear stage, when the cells are still weakly differentiated but still retain totipotency, are most susceptible to androgenesis [3, 27-28]. At the same time, overmature microspores lose their activity in morphogenesis, and, conversely, those that are too young are not yet able to respond to induction signals [1, 3, 18, 20, 22, 26-27].

Chemical Factors

The composition of the nutrient medium plays a central role in the regulation of androgenesis. The most important components are the mineral composition of the medium, particularly nitrogen sources (ammonium and nitrate), trace elements, and the concentration and combination of phytohormones. An optimal balance between these substances has a critical impact on callus induction and the development of regenerated plants. The most widely used basic nutrient media and their modifications include Murashige and Skoog (MS), Gamborga B-5, N6, and others, which demonstrate high efficiency for various rice genotypes [1, 3, 18, 20, 29]. Carbohydrates are used as an energy source and osmotic agent, and the type of source chosen directly influences an-

ther response [28]. Literature data indicate that maltose is more effective in anther culture than sucrose, which can have a toxic effect when broken down into glucose and fructose [27, 30]. In addition, organic additives such as yeast extracts, casein hydrolysate, amino acids (glutamine, proline), and vitamins (thiamine, inositol) affect the acceleration of cellular metabolic activity and morphogenesis [3, 18, 20]. For work with sensitive varieties, as well as to prevent necrosis and browning, antioxidants and phenol adsorbents (ascorbic acid, PVP, $AgNO_3$) are added to the nutrient medium [22, 29]. Agar is mainly used as a gelling agent in solid nutrient media; however, some studies have noted that when agar was replaced with Gelrite, starch increased the efficiency of androgenesis in vitro [27, 31].

Physical factors

Physical conditions significantly influence the effectiveness of androgenesis. Anthers can be cultured on both solid and liquid nutrient media [3, 18, 20]. However, it has been noted that combined and two-phase media promote nutrient diffusion and improve aeration, which directly impacts embryo development [22, 29]. Stress pretreatment is most commonly used in rice anther culture, primarily cold treatment at temperatures ranging from +4 to +10 °C for several days; less commonly, heat treatment at +33-35 °C, as well as a combination of both, is used. Osmotic stress, such as sucrose starvation, radiation, and electrical stimulation, is also introduced [3, 18, 20, 22, 29]. Light and temperature conditions are also important for microspore cultivation, influencing photomorphogenesis and regeneration [32–34]. Therefore, during the initial stages, cultures are incubated in the dark and then transferred to a photoperiod at a temperature of 25–28 °C, which stimulates seedling formation. This range of treatments is necessary to induce the process of microspore reprogramming to sporophytic development [35-36].

In summary, the success of anther culture depends on the careful selection of all the above parameters, taking into account the specific rice genotype. Only a comprehensive approach to the physical, chemical, and biological components will increase the efficiency of androgenesis and guarantee a high regeneration rate of green, viable plants [37–39].

Anther culture specifics in different rice subspecies

The specific subspecies of rice from which the source material belongs is a critical factor influencing the success of androgenesis. Although the japonica and indica subspecies share a common genetic foundation, their distinct responses to anther culture are explained by differences in the physiological and biochemical characteristics of these subspecies. It has been noted that japonica subspecies varieties exhibit stable callus formation and high responsiveness, as well as a high percentage of regenerated plants, making them a model for androgenesis [40-41]. Meanwhile, the indica subspecies exhibits pronounced recalcitrance, i.e., a low capacity for callus formation, weak embryogenic competence, and an extremely high incidence of albino plants. These difficulties are complex in nature and are associated with the expression characteristics of heat shock genes (HSPs), stress response proteins, and antioxidant defense enzymes, which determine the successful development of microspores from the gametophyte to the sporophyte. The indica subspecies is also characterized by high activity of polyphenoloxidases, which affect the browning of calli and anthers themselves, which in turn causes necrosis and the accumulation of phenolic compounds that have a detrimental toxic effect [42]. The main goal of haploid technology was to overcome recalcitrance. It is known from the literature that the most effective intersubspecific hybrids (F_1 indica × japonica), possessing combined embryogenic competence, turned out to be the first generation; the use of stress treatments (short-term cold shock at 8–10 °C, osmotic stress, sucrose starvation), aimed at activating the sporophytic development program; The replacement of sucrose with maltose as a more stable carbon source; and the addition of silver nitrate ($AgNO_3$) to suppress tissue browning [43, 44]. Significant progress has been achieved using various modifications to nutrient media, including enrichment with casein hydrolysate, glutamines, and ammonium and potassium salts. The use of isolated microspore culture has proven to be the most promising, increasing the ability to control the conditions for embryogenesis and eliminating the influence of somatic tissues [45].

Efficiency of the anther culture method in applied rice breeding

Anther culture technology has proven to be an effective tool for enhancing breeding, enabling the production of homozygous rice lines in a single generation and shortening the breeding cycle. Over the past decades, the method has been successfully implemented in practice, as evidenced by the development of new rice varieties worldwide. Back in the 1980s, Chinese scientists developed the “Huayu 15” variety, which yielded 8–11 t/ha. It was created from a haploid F-1 hybrid plant through anther culture followed by double haploidization. This variety demonstrated that anther culture enables heterosis while maintaining stability and uniformity in the offspring. Since then, dozens of highly productive rice varieties have been developed in China through anther culture [41]. These plants are resistant to a range of diseases, are high-yielding, and

are characterized by high-quality grain. By 2015, more than 100 rice lines and varieties had been bred in China. Therefore, China is a striking example of the large-scale implementation of androgenic technology in rice breeding. Domestic experience describes the development of the early-ripening rice variety "Fatima", obtained by breeders at the I. Zhakhaev Kazakh Research Institute of Rice Growing. This variety was obtained through anther culture from the mutant IRRI line (Dihaloid Ko 293), which allowed for the retention of valuable traits in the homozygous state. In state trials, "Fatima" demonstrated a yield increase of 6–10 % compared to the standard in various growing regions. Furthermore, the variety exhibits good resistance to lodging, grain shattering, pests, and diseases. "Fatima" was one of the first Kazakh rice varieties to clearly demonstrate the effectiveness of anther culture for the accelerated development of new competitive varieties [46]. These examples confirm that the inclusion of anther culture in breeding programs significantly accelerates the development of genetically uniform lines and new rice varieties with improved properties [47].

Challenges in Rice Anther Cultivation

Rice anther culture in breeding allows for shorter cycles and increased hybridization efficiency. Furthermore, this method is easily integrated into transgenesis using molecular markers, enabling the development of new varieties with multiple resistances, adapted to various conditions, superior quality, and high yields (Tab. 1). However, the practical use of rice anther culture still faces a number of challenges that limit its full potential.

Table 1

Rice varieties obtained from anther culture

Variety	Characteristics	Country	Links
Danfeng 1	High grain quality, high yield	China	[48]
Zhonghua 8, Zhonghua 9	Blast resistance	China	[49]
Zhonghua 10	High grain quality, salt tolerance	China	[50]
1647S	High yield	China	[51]
Huageng 45	Salt tolerance, lodging resistance, fire blight resistance, moderate resistance to anthracnose, cercospora leaf spot, and false smut	China	[52]
Hejiang 21, Longgeng 1, Longgeng 3, Longgeng 4, Longgeng 7, Longgeng 8	Blast resistance, high grain quality, high yield	China	[53]
Jiudao 26	Moderately resistant to leaf scab, moderately susceptible to panicle scab, high grain quality	China	[54]
Zhonghua 15	Resistance to fire blight and blast, high yield	China	[55]
Huageng 15	Salt tolerance	China	[56]
Zhonghua 14, Zhonghua 16	Salt tolerance, lodging resistance, drought tolerance	China	[57, 58]
Longgeng 10, Longgeng 12	Blast resistance, high quality	China	[59, 60]
Huayu 13	Resistance to blast, leaf rot, and false smut, high grain quality, good flavor, high yield	China	[61]
Huayu 15	Lodging and disease resistance, good grain quality	China	[62]
HD27	High grain quality, resistance to diseases, early flowering	China	[63]
Chongshang 2022	Scab and lodging resistance, good grain quality	China	[64]
Shuhui 162	Scab resistance, high grain quality	China	[65]
Hua 1B	Good crossing characteristics, high combining ability	China	[66]
Hua 2B	High grain quality	China	[67]
Hua 03	High protein content (13.7 %)	China	[68]
Chuanhui 907		China	[69]
Chuanhui 1618	High grain quality, blast resistance	China	[70]
Miai 64S	Large panicle, high grain quality, blast resistance	China	[71]
1103S, 8906S, 8902S	High yield	China	[72]
Liangyou 1178	Stable sterility, practical significance for the breeding process	China	[72]

Continuation of Table 1

Variety	Characteristics	Country	Links
HS-1, HS-2, HS-3	High yield, high grain quality, multifactorial resistance	China	[73]
Hua 1A	Good crossing characteristics, high combining ability	China	[74]
1286S, 6442S	Good crossing characteristics, high combining ability	China	[75]
Jinshan S-1	High yield	China	[76]
Huaxiang 7	Stable sterility, practical significance for the breeding process	China	[77]
Xiang 125S	High yield, moderate blast resistance	China	[78]
Hua 2A	High grain quality	China	[79]
V25S	Stable sterility, practical significance for the breeding process	China	[80]
EH1S	High seed set percentage Crossbreeding, high grain quality	China	[81]
Guan 18, Gan Xhao Xian 11	High seed set rate in crossbreeding, blast resistance	China	[82]
Huayu 15	Early maturity, disease resistance	China	[62]
Huayu I, Huayu II, Xin Xiu, Late Keng 959, Tunghua 1, Tunghua 2, Tunghua 3, Tanghuo 2, Huajian 7902, Zhonghua 9	Resistance to lodging and diseases, good grain quality	China	[68]
Milyang 90	High yield, high grain quality, resistance to blast and bacterial blight	China	[83]
CR Dhan 10 (CRAC2221–43), Satyakrishna	Good grain quality, resistance to brown leafhopper and stripe virus	India	[84]
CR Dhan 801 (CRAC2224–1041, IET18720), Phalguni	Resistance to leaf rot and stem borer	India	[85]
Janka	Resistance to blast and gall midge, moderate resistance to stripe virus, yellow stem rust, and brown spot	India	[86]
Abel	Drought resistance, good grain quality	India	[86]
Parag 401	Cold tolerance	India	[87]
Risabell	High grain quality, resistance to chlorosis caused by iron deficiency	India	[86]
Hwacheongbyeo, Joryeongbyeo, Hwajinbyeo	High grain quality, good taste	South Korea	[88]
PSBRc 50 Bycol (IR 51500-AC11-1)	Resistance to brown leafhopper, stripe virus, blast, and bacterial blight Scald	South Korea	[89]
Privolny-4	Salt-tolerant	Russia	[41]
Sonnet	Blast-resistant, high yield, does not require high fertilizer doses	Russia	[41]
Bicol (IR51500AC11–1)	Can be grown under various irrigation regimes, does not shatter when overripe, good flavor	Philippines	[90]
AC-1	Salt-tolerant	Bangladesh	[91]
Joiku 394, Hirohikari, AC. No.1, Hirohonami, Kibinohana	Salt-tolerant	Japan	[92]
Dama	Cold-tolerant	Hungary	[93]
Fatima	High yield, blast-resistant, good flavor	Kazakhstan (Institute of Plant Biology and Biotechnology)	[94]

The key issue in rice anther culture breeding is genotype, as it largely determines the effectiveness of the method. Current breeding programs face numerous challenges, including lengthy stages, a high workload, low callus formation rates, low green shoot regeneration rates, and low androgenesis efficiency in rice

of the indica subspecies. To mitigate genotype limitations, previous studies have suggested selecting parent materials with high anther culture efficiency or using indica-japonica hybrid progeny with a higher proportion of japonica ancestry to exploit heterosis and improve anther culture efficiency. Furthermore, identifying genes that influence anther culture efficiency and using transgenesis with molecular markers can alleviate genotype limitations.

The occurrence of albino plants, as well as browning of callus and anthers, negatively impacts androgenesis efficiency. Albinism is a recessive trait controlled by a group of loci of abnormal gene expression or mutations; the absence of chloroplasts is influenced by both genes and environmental factors [95]. Browning occurs upon activation of polyphenoloxidases produced in tissues and leading to inactivation of other enzymes, which inhibits growth [96]. Any anther is susceptible to browning, regardless of age, and high concentrations of inorganic salts and sugars in the nutrient medium can accelerate this process. An effective strategy for preventing these two phenomena may be the selection of young spikelets whose anthers are still mononuclear, regulation of environmental parameters (light, temperature), and the use of various antioxidants (activated carbon, PVP, vitamin C, and $Na_2S_2O_3$) [97]. In another case, it was shown that insertion of a transposon into the BOC1 gene promoter reduces callus browning in culture [98]. Literature data have shown that reducing the manganese content and the concentration of inorganic salts in the callus induction medium, as well as optimizing the concentration of hormones (e.g., KT or 2,4-D) can reduce the formation of albino plants and simultaneously increase the callusogenesis rates [99].

QTLs associated with antheric cultivability in rice

The ability of rice microspores to develop in culture is a complex quantitative trait controlled by a large number of genes. Furthermore, genotypic differences depend on the rice subspecies. Literature data have shown that QTL mapping methods have yielded a number of loci that influence key stages of androgenesis [100]. Experiments with a DH population (double haploids) from a cross between the indica × japonica subspecies revealed QTLs associated with callus formation frequency located on chromosomes 6, 7, 8, 10, and 12, as well as those for the ability to regenerate green plants on chromosomes 1 and 9, respectively. A QTL responsible for the formation of albinos was also found on chromosome 9. In another study, a QTL associated with green plant regeneration was mapped on chromosome 10 and used to select plants with enhanced regeneration capacity in the Milyan 23 × Gihobyeo rice population [101]. As a result, the marker associated with this locus was integrated into the MAS program to obtain a new rice line with enhanced regeneration capacity, saving time and resources for obtaining DH forms. Despite the difficulties of phenotyping, which limit the number of QTL in androgenesis, the use of modern methods, in particular Segregation Distortion Analysis, provides new opportunities for mapping the corresponding loci. Using this method, five loci were identified: SDL1.1, SDL1.2, SDL2, SDL5, SDL7 (2023), the alleles of which were successfully transmitted to DH progeny with increased frequency, indicating successful microspore development *in vitro* [102].

Prospects for the development of anther culture—new technologies and integrated approaches

Rice anther culture is a highly promising method with enormous potential for accelerating rice breeding. Isolated microspores are easily cryopreserved, which is useful for long-term storage of haploid material. However, anther culture remains a time-consuming and labor-intensive method, which poses a significant challenge to scalability. Future advances anticipate the introduction of high-throughput automated systems. Advanced molecular breeding approaches are actively integrating anther culture with high-precision genetic tools, such as genome editing technologies (CRISPR/Cas9) and marker-assisted selection (MAS) [103]. Combining androgenesis with MAS offers expanded opportunities for breeding new varieties and increases the accuracy of early-stage selection. Selecting hybrids using molecular markers followed by fixation of the desired genotype using anther culture creates conditions for the rapid production of homozygous lines containing desired alleles for quality, resistance, or productivity. This approach has already proven successful in a number of breeding programs, for example, in the development of early-ripening, high-yielding Chinese varieties. The effectiveness of the MAS method is also reflected in the screening of DH lines, allowing for the rapid identification of promising forms, bypassing years of field trials. In a more technologically advanced perspective, rice anther culture can be combined with genome editing methods. This can be achieved by editing parental forms or by directly delivering CRISPR/Cas9 complexes to embryogenic calli or microspores. This makes it possible to introduce the necessary point mutations and immediately obtain homozygous plants without lengthy selection. Furthermore, editing genes that limit the efficiency of androgenesis

itself, particularly those responsible for albinism, opens a new avenue for significantly increasing the effectiveness of the technology itself. Thus, introducing MAS and CRISPR/Cas9 tools into anther culture allows for the dramatic acceleration of the selection process, improvement of the source material, and the capture of valuable traits [104, 105]. All of this makes androgenesis a crucial element of the modern biotechnological platform in rice cultivation.

Conclusion

Anther culture is increasingly integrated into modern breeding strategies as a tool for "accelerated breeding", revolutionizing rice breeding by significantly accelerating the development of new lines and varieties. The use of anther culture allows for the reduction of the breeding cycle for a new variety from 6–8 generations to 2-3, which is particularly important for strategically important crops such as rice. Anther culture makes it possible to quickly respond to production needs, from improving yield and grain quality to introducing disease resistance genes. Combining this method with marker-assisted selection and genome editing (MAS, CRISPR/Cas9) enables the targeted production of homozygous lines with specific sets of valuable traits in a single generation. In China, over 200 commercial rice varieties have been developed through anther culture, many of which have occupied significant acreage and contribute to food security. In Vietnam, India, Bangladesh, and other Asian countries, haploid technologies are also used for the accelerated improvement of local varieties (improving quality, disease resistance, and producing lines for hybrid breeding). Kazakhstan's experience with the Fatima variety demonstrates the method's effectiveness even in the harsh continental climate of Central Asia. Thus, the method serves as a cornerstone of modern breeding programs, complementing traditional hybridization and integrating with molecular and genomic technologies.

The responsiveness of anthers and microspores cultured in vitro is influenced by many interacting factors: growing conditions of the donor plants, genotype, physiological state of the donor plant, stage of microspore development, anther pretreatment, and the composition of the nutrient medium. To improve the efficiency of producing haploids and dihaploids, it is necessary to conduct a comprehensive study to evaluate the structural features and physiological mechanisms of the anther cell reprogramming process and their responsiveness to cultivation conditions. The development and use of new rice varieties that are resistant to pests, diseases, and drought, as well as tolerant of saline-alkaline environments, high-yielding, and of superior quality, can be facilitated by integrating anther culture with other technologies, such as transgenic technology, molecular marker-assisted selection, and CRISPR/Cas9 gene editing. Standardizing and simplifying anther culture protocols to minimize the impact of experimental operations, along with improving the anther culture system in rice, will facilitate the development and use of new rice varieties and enrich rice germplasm resources in Kazakhstan. Anther culture in rice has evolved from basic experimental protocols to high-tech breeding systems. Historical successes and ongoing challenges (recalcitrance, albinism, variability) are supported by modern technological solutions, making this method an effective tool for modern rice improvement programs.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript: **Usenbekov B.N.** — conceptualization, data analysis, investigation; **Mukhambetzhanov S.K., Kurbangaliyeva T.A., Amirova A.K.** — data analysis, writing draft; **Sartbayeva I.A., Kirshibaev E.A., Gabdullina Ye.Zh., Yerezhepov D.A., Yerezhepov A.E.** — data curation, data collection, draft writing.

References

1 Tripathy, S. K., Lenka, D., Prusti, A. M., Mishra, D., Swain, D., & Behera, S. K. (2019). Anther culture in rice: Progress and breeding perspective. *Applied Biological Research, 21*(2), 87. https://doi.org/10.5958/0974-4517.2019.00012.0

2 Silva, T. D. (2010). Indica rice anther culture: can the impasse be surpassed? *Plant Cell, Tissue and Organ Culture (PCTOC), 100*(1), 1–11.

3 Mishra, R., & Rao, G. J. N. (2016). In-vitro androgenesis in rice: advantages, constraints and future prospects. *Rice Science, 23*(2), 57–68.

4 Mayakaduwa, D. M. R. G., & Silva, T. D. (2018). Anther culture as a supplementary tool for rice breeding. *Rice Crop-Current Developments*, 1–16.

5 Ali, J., Nicolas, K. L. C., Akther, S., Torabi, A., Ebadi, A. A., Marfori-Nazarea, C. M., & Mahender, A. (2021). Improved anther culture media for enhanced callus formation and plant regeneration in rice (*Oryza sativa* L.). *Plants, 10*(5), 839.

6 Islam, M. T., Arif, M. R., Hasan, M. T., & Robin, A. H. K. (2023). Anther culture in crop plants: progress and perspectives. *Plant Breeding and Biotechnology, 11*(2), 69–96.

7 Morinaga, T., & Fukushima, E. (1931). Preliminary report on the haploid plant of rice, *Oryza sativa* L. *Proceedings of the Imperial Academy, 7*(10), 383-384.

8 Nakamura, S. (1933). The haploid plant in rice. *Jap. Jour. Gen, 8*, 223–227.

9 Niizeki, H., & Oono, K. (1968). Induction of haploid rice plant from anther culture. *Proceedings of the Japan Academy, 44*(6), 554–557.

10 Guha, S., Iyer, R. D., Gupta, N., & Swaminathan, M. S. (1970). Totipotency of gametic cells and the production of haploids in rice. *Current Science, 39*(8), 174–176.

11 Chu, C. (1975). Establishment of an efficient medium for another culture of rice through comparative experiments on the nitrogen sources. *Scientia Sinica, 18*, 223–231.

12 Chen, C. C. (1977). In vitro development of plants from microspores of rice. *In vitro, 13*(8), 484–489.

13 Hu, H., & Zeng, J. Z. (1984). Development of new varieties via anther culture. *Handbook of plant cell culture, 3*, 65–90.

14 Niizeki, H., & Oono, K. (1968). Induction of haploid rice plant from anther culture. *Proceedings of the Japan Academy, 44*(6), 554–557.

15 Zapata, F. J. (1985). Rice anther culture at IRRI. *Biotechnology in International Agriculture Research*. IRRI, 85–89.

16 Tripathy, S. K., Swain, D., Mohapatra, P. M., Prusti, A. M., Sahoo, B., Panda, S., & Behera, S. K. (2019). Exploring factors affecting anther culture in rice (Oryza sativa L.). *Journal of Applied Biology and Biotechnology, 7*(02), 87–92.

17 Loo, S. W., & Xu, Z. H. (1991). Anther culture for rice improvement in China. *In Rice*, 151–159. Berlin, Heidelberg: Springer Berlin Heidelberg.

18 Kyum, M., Kaur, H., Kamboj, A., Goyal, L., & Bhatia, D. (2022). Strategies and prospects of haploid induction in rice (Oryza sativa). *Plant Breeding, 141*(1), 1–11.

19 Bukharbayeva, A. Zh., Akimbekova, G. U., & Kerimbek, G. E. (2023). Rice growing in Kazakhstan: internal reserves and competitiveness. *Problems of AgriMarket, 1*, 123–130. https://doi.org/10.46666/2023-1.2708-9991.14

20 Lantos, C., Jancsó, M., Székely, Á., Szalóki, T., Venkatanagappa, S., & Pauk, J. (2023). Development of in vitro anther culture for doubled haploid plant production in indica rice (*Oryza sativa* L.) genotypes. *Plants, 12*(9), 1774.

21 Herath, H. M. I., & Bandara, D. C. (2011). Anther culture performance in selected high yielding indica (of Sri Lanka) and japonica rice varieties and their inter sub-specific hybrids. *Journal of the National Science Foundation of Sri Lanka, 39*(2).

22 Patra, S., Mohanty, P., & Tripathy, S. K. (2019). Scope of androgenesis for genetic improvement in rice. *Emergent Life Sciences Research, 5*, 23–28.

23 Guo, G., Liu, S., Zhang, S., Yang, L., Zong, Y., Halford, N. G., & Chen, Z. (2024). Generic Workflow of a Highly Effective and Easy Anther Culture Method for Both Japonica and Indica Rice. *Plants, 13*(17), 2531.

24 Raina, S. K., & Zapata, F. J. (1997). Enhanced anther culture efficiency of indica rice (Oryza sativa L.) through modification of the culture media. *Plant breeding, 116*(4), 305–315.

25 Silva, T. D., & Ratnayake, W. J. (2009). *Anther culture potential of indica rice varieties, kuruluthuda and Bg 250*.

26 Mayakaduwa, D. M. R. G., & Silva, T. D. (2017). A cytological indicator allows rapid assessment of microspore maturity, leading to improved in vitro anther response in Indica rice (*Oryza sativa* L.). *In Vitro Cellular & Developmental Biology-Plant, 53*(6), 591–597.

27 Mayakaduwa, D. M. R. G., & Silva, T. D. (2018). Anther culture as a supplementary tool for rice breeding. *Rice Crop-Current Developments*, 1–16.

28 Germana, M. A. (2011). Anther culture for haploid and doubled haploid production. *Plant Cell, Tissue and Organ Culture (PCTOC), 104*(3), 283–300.

29 Tripathy, S. K. (2018). Anther culture for double haploid breeding in rice-a way forward. *Rice Genomics and Genetics, 9.*

30 Lentini, Z., Reyes, P., Martínez, C. P., & Roca, W. M. (1995). Androgenesis of highly recalcitrant rice genotypes with maltose and silver nitrate. *Plant Science, 110*(1), 127–138.

31 Gill, R., Kaur, N., Sindhu, A. S., Bharaj, T. S., & Gosal, S. S. (2003). Improved methods for anther and pollen culture in rice. *Advances in rice genetics, 15*, 503.

32 Matsushima, T., Kikuchi, S., Takaiwa, F., & OoNo, K. (1988). Regeneration of plants by pollen culture in rice (Oryza sativa L.). *Plant tissue culture letters, 5*(2), 78–81.

33 Gupta, H. S., & Borthakur, D. N. (1987). Improved rate of callus induction from rice anther culture following microscopic staging of microspores in iron alum-haematoxylin. *Theoretical and Applied Genetics, 74*(1), 95–99.

34 Gao, R., Zong, Y., Zhang, S., Guo, G., Zhang, W., Chen, Z., & Li, Y. (2024). Efficient isolated microspore culture protocol for callus induction and plantlet regeneration in japonica rice (*Oryza sativa* L.). *Plant Methods, 20*(1), 76.

35 Lee, S. T., & Huang, W. L. (2014). Osmotic stress stimulates shoot organogenesis in callus of rice (*Oryza sativa* L.) via auxin signaling and carbohydrate metabolism regulation. *Plant growth regulation, 73*(2), 193–204.

36 Raina, S. K., & Irfan, S. T. (1998). High-frequency embryogenesis and plantlet regeneration from isolated microspores of indica rice. *Plant Cell Reports, 17*(12), 957–962.

37 Shariatpanahi, M. E., Bal, U., Heberle-Bors, E., & Touraev, A. (2006). Stresses applied for the re-programming of plant microspores towards in vitro embryogenesis. *Physiologia Plantarum, 127*(4), 519–534.

38 Chen, Q. F., Wang, C. L., Lu, Y. M., Shen, M., Afza, R., Duren, M. V., & Brunner, H. (2001). Anther culture in connection with induced mutations for rice improvement. *Euphytica, 120*(3), 401–408.

39 Mkuya, M. S., Hua-min, S. I., Wen-zhen, L. I. U., & Zong-xiu, S. U. N. (2005). Effect of 137Cs gamma rays to panicles on rice anther culture. *Rice Science, 12*(4), 299.

40 Mayakaduwa, D. M. R. G., & Silva, T. D. (2018). Anther culture as a supplementary tool for rice breeding. Rice Crop-*Current Developments*, 1–16.

41 Silva, T. D. (2010). Indica rice anther culture: can the impasse be surpassed. *Plant Cell, Tissue and Organ Culture (PCTOC), 100*(1), 1–11.

42 Chen, X., Li, S., Zha, W., Li, C., Zhou, L., You, A., & Wu, Y. (2025). Advances in Anther Culture-Based Rice Breeding in China. *Plants, 14*(11), 1586.

43 Kanaoka, Y., Kuniyoshi, D., Inada, E., Koide, Y., Okamoto, Y., Yasui, H., & Kishima, Y. (2018). Anther culture in rice proportionally rescues microspores according to gametophytic gene effect and enhances genetic study of hybrid sterility. *Plant methods, 14*(1), 102.

44 Rice variety Fatima. Retrieved from https://baibolsyn.kz/ru/semena/ris-variety-fatima/

45 (2016). *Catalogue of rice and vegetable and melon varieties of Kuban selection.* Krasnodar: EDVI.

46 Lantos, C., Jancsó, M., Székely, Á., Nagy, É., Szalóki, T., & Pauk, J. (2022). Improvement of anther culture to integrate doubled haploid technology in temperate rice (Oryza sativa L.) breeding. *Plants, 11*(24), 3446.

47 Rout, P., Naik, N., Ngangkham, U., Verma, R. L., Katara, J. L., Singh, O. N., & Samantaray, S. (2016). Doubled Haploids generated through anther culture from an elite long duration rice hybrid, CRHR32: Method optimization and molecular characterization. *Plant Biotechnology, 33*(3), 177–186.

48 China Rice Data Center. Retrieved from https://ricedata.cn/variety/varis/613663.htm

49 Li, M. (1983). New rice varieties Zhonghua No.8 and No.9. *Bull. Agric. Sci. Technol., 3*(7), 9.

50 Li, M. (1988). Rice cultivar — Zhonghua 10. *Bull. Agric. Sci. Technol., 1*, 26.

51 Liu, J., Hu, D., Hong, L., Liu, B., Mao, Z., Li, Z., & Zhang, A. (1995). Breeding of photothermosensitive male sterile lines of japonica rice by flower culture technique. *Beijing Agric. Sci., 4*, 22–24.

52 Er, D. (1996). A new rice pollen cultivation-Huajing 45. *Mod. Agric., 10*, 11.

53 Jiang, J., Jin, C., Hou, C., Jin, X., & Yang, B. (2001). Progress in research and application of rice anther culture. *Chin. Agric. Sci. Bull., 4*, 49–52.

54 Jin, S., Jiang, H., Li, X., & Zhang, W. (2001). Breeding of the new rice variety Jiudao 26 (Jiuhua 3). *Jilin Agric. Sci., 4*, 27-28.

55 Zhonghua 15. ICS, CAAS. 1 January 2002.

56 China Rice Data Center. Retrieved from https://ricedata.cn/variety/varis/605929.htm

57 Li, M. (2000). Rice variety Zhonghua 14 (93-108). *China Rural. Sci. Technol., 7*, 21.

58 Li, M., Zhang, L., Lin, Z., Zhang, Q., Kuang, B., & Li, W. (2003). Breeding of new rice variety Zhonghua 16. *Crops, 4*, 50.

59 Guan, S. (2000). Breeding and High Yield Cultivation Model of Longjing 10, a New Rice Flower Cultivation Variety. *Chin. Agric. Sci. Bull., 3*, 75-76.

60 Zhang, S. (2003). Characteristics and high-yield cultivation techniques of a new rice variety Longjing 12. *Crops, 6*, 37.

61 Li, Y., Zou, M., Sun, H., Niu, J., Wang, J., & Liang, Y. (2007). Breeding of new japonica rice variety Huayu 13 with high quality and high yield. *Crop Res., 3*, 285-286.

62 Shouyi, L., & Shouyin, H. (1991). Huayu 15, a high yielding rice variety bred by anther culture. In *Rice*, 230–247. Berlin, Heidelberg.

63 Ye, J., Ye, H., Zhai, R., Zhu, G., & Zhang, X. (2020). Zhejing 7A, a japonica rice sterile line bred by anther culture technology. *J. Zhejiang Agric. Sci., 61*, 1529–1530.

64 Huang, W., Yan, Y., Wang, K., Zhan, L., Hu, Z., & Wu, S. (2022). Breeding of a new japonica rice variety with fragrance and soft by anther culture technique and molecular marker-assisted selection method. *Acta Agric. Shanghai, 38*, 41–46.

65 (2001). Rice Restorer Line Shuhui 162. RRI, SAU. Retrieved from https://kns.cnki.net/kcms2/article/abstract?v=fSCzX0TVvUjHZGOyyaYAw_XuOwJaRwfQLbTyYFiwxe5DzlxsZTItMQ_4bKb_CQs5GVNjmOUU1H_n51O29VlFJVrOrfofP2gHG56smMmSSe1Fol3KurlECG2CgCVycaal-ywP9CQnRHMwC4C7EF6R7FT0gg-ysuF-EAfvPkMa2FX1X_-23eAWcw==&uniplatform=NZKPT&language=CHS

66 Li, W., Chen, Q., Pan, R., Zhang, S., Qi, J., Lin, G., Jiang, S., Lin, L., Ji, B., & Zheng, X. (1998). Breeding of rice cytoplasmic male sterile line 1A. *J. Fujian Agric. For. Univ., 2*, 2–5.

67 Pan, R., Zheng, B., Mao, D., Guan, H., Chen, Z., Lin, L., Zhao, J., & Zhou, Y. (2009). Breeding of Quality Rice CMS Line Hua 2 A by Anther Culture. *Hybrid. Rice, 24*, 12–15.

68 Yang, X.R., & Fu, H.H. (1989). A high protein Indica rice. *International Rice Research Newsletter, 14*(3), 14-15.

69 Wang, P., Xiang, Y., Zhang, Z., Wang, M., Cai, P., & Zhang, Z. (2010). Breeding and Utilization of Indica Restorer Line Chuanhui 907 with High Combining Ability. *Hybrid Rice, 25* (Suppl. S1), 195–197.

70 Wang, P., Zhang, Z., Xiang, Y., Wang, M., Cai, P., Zhang, Z., & Lin, Y. (2012). Breeding and Utilization of Indica Restorer Line Chuanhui 1618 with Good Grain Quality and High Combining Ability. *Hybrid Rice, 27*, 20–22.

71 Li, X., Pan, X., Chen, Z., Yu, H., Chen, Z., & Gu, M. (1995). Anther culture of photosensttive male sterile rice and the breeding of miai64s. *J. Yangzhou Univ. (Agric. Life Sci. Ed.), 4*, 7–12.

72 Li, W., Chen, Q., Qi, J., Pan, R., Jiang, S., Ji, B., Lin, G., Lin, L., Zhou, Y., Wu, R., & et al. (1996). Breeding report of photosensitive male sterile line Hs-1 in indica rice. *J. Fujian Agric. For. Univ. (Nat. Sci. Ed.), 3*, 4–10.

73 Li, W., Chen, Q., Pan, R., Zhang, S., Qi, J., Lin, G., Jiang, S., Lin, L., Ji, B., & Zheng, X. (1998). Breeding of rice cytoplasmic male sterile line 1A. J. *Fujian Agric. For. Univ. (Nat. Sci. Ed.), 2*, 2–5.

74 (2000). *Research on the Breeding of Indica Dual-Purpose Nuclear Sterile 6442S and 1286S*. RRI, JAAS.1.

75 Chen, Z., Lin, L., Zhou, Y., Pan, R., Mao, D., Wu, J., & Guan, H. (2005). Breeding of the Low-temperature Sensitive Genic Male Sterile Line, Jinshan S-1 by means of Anther Culture. *Acta Agric. Univ. Jiangxiensis, 5*, 648–652.

76 Pu, Z., Xiang, Y., Zhang, Z., Cai, P., Zhou, X., & Zhang, Z. (2007). Huaxiang 7, a NewIndica Hybrid Rice Combination. *Hybrid Rice, 5*, 85-86.

77 Feng, S., Jiang, X., Zhao, S., Hou, H., & Xiao, G. (2008). A Preliminary Study on Genetic Homogenization of Thermosensitive Genic Male Sterile(TGMS) Line Xiang 125S in Rice through Anther Culture. *Hybrid Rice, 4*, 62–64.

78 Pan, R., Zheng, B., Mao, D., Guan, H., Chen, Z., Lin, L., Zhao, J., & Zhou, Y. (2009). Breeding of Quality Rice CMS Line Hua 2 A by Anther Culture. *Hybrid. Rice, 24*, 12–15.

79 Xu, X., Xun, F., Ma, G., Zhou, H., Wang, C., Zhang, H., & Li, S. (2018). Breeding of Rice PTGMS Line V25S by Anther Culture. *Hybrid Rice, 33*, 10–12.

80 Liu, K., Li, S., Xu, H., Chen, Z., Yang, G., & You, A. (2019). Breeding of high-quality two-line sterile rice line EH1S using anther culture technology. *Rural. Econ. Sci. -Technol., 30*, 48-49.

81 (2007-2008). *Central Rice Research Institute (CRRI) Annual Report*. Retrieved from https://icar-nrri.in/annual-report/

82 Chung, G.S. (1987). Application of anther culture technique for rice (*Oryza sativa* L.) improvement. *In: Korea-China Plant Tissue Culture Symposium.* Beijing, China: Academia Sinica Publishers, 36–56.

83 (2008-2009). Central Rice Research Institute (CRRI) Annual Report. Retrieved from https://icar-nrri.in/annual-report/

84 (2009-2010). Central Rice Research Institute (CRRI) Annual Report. Retrieved from https://icar-nrri.in/annual-report/

85 Pauk, J., Janeso, M., & Simon-Kiss, I. (2009). Rice doubled haploids and breeding. In*: Advances in Haploid Production in Higher Plants*, Springer, Amsterdam, Netherlands, 189–197.

86 Patil, V.D., Nerkar, Y.S., Misal, M.B., & Harkal, S.R. (1997). Parag 401, a semidwarf rice variety developed through anther culture. *International Rice Research Newsletter*, *22*(2), 19.

87 Lee, Y.T., Lim, M.S., Kim, H.S., Shin, H.T., Kim, C.H., Bae, S.H., & Cho, C.I. (1989). An anther derived new high quality variety with disease and insect resistance 'Hwacheongbyeo'. *Research Report — Rural Development Administration Rice, 31*(2), 27–34.

88 88. Senadhira, D., Gregorio, G.B., Mendoza, R.D., Roxas, J.P., Alejar, M.S, Reyes, R.Y., Patena, C.C., DelaCruz H.C., Padolina, T.F., Galvez, A.M., & Gamiao, B.P. (1996). *New Rice Varieties that Defy Soil Salinity*. University of the Philippines, Los Baños, Philippines.

89 Senadhira, D., Zapata-Arias, F.J., Gregorio, G.B., Alejar, M.S., Cruz, H.C.D.L., & Padolina, T.F. (2002). Development of the first salt-tolerant rice cultivar through indica/indica anther culture. *Field Crops Res., 76*, 103–110.

90 Thomson, M.J., Ocampo, M.D., Egdane, J., Rahman, M.A., Sajise, A.G., Adorada, D.L., Tumimbang-Raiz, E., Blumwald, E., Seraj, Z.I., & Singh, R.K. (2010). Characterizing the saltol quantitative trait locus for salinity tolerance in rice. *Rice, 3*, 148–160.

91 Singh, R.B. (1998). Agricultural biotechnology in Asia-Pacific region. *In: Agricultural Biotechnology in the Developing World. FAO Research & Technology Development Division*, 53–55. Daya Publishing House, New Delhi, India.

92 Heszky, L.E., & Simon-Kiss, I. (1992). The first plant variety of biotechnology origin in Hungary, registered in 1992. *Hungarian Agricultural Research, 1*, 30–32.

93 (2025). SortCom. (n.d.). Rice varieties. Retrieved from https://sortcom.kz/rice/

94 Ma, H. (2019). Study on the Characteristics of Rice Albino Seedlings Induced by in Planta of Rice. *Thesis of Master's Study on the Characteristics of Rice Albino Seedlings Induced by in Planta of Rice.*

95 Zeng, L., & Liu, Y. (2007). Research progress on browning in plant tissue culture. *Anhui Agric. Sci. Bull., 14*, 49-50.

96 Zhang, X. (2018). Study on the inhibiting effect of several anti-browning agents on the browning phenomenon of pistachio seed embryo culture. *Sci. Technol. Tianjin Agric. For, 1*, 7-8.

97 Zhang, K., Su, J., Xu, M., Zhou, Z., Zhu, X., Ma, X., & Fu, Y. (2020). A common wild rice-derived BOC1 allele reduces callus browning in indica rice transformation. *Nature Communications, 11(1)*, 443.

98 He, M., Song, D., Zhang, L., & Huang, S. (2010). Common problems and prevention measures in rice anther culture. *North Rice, 40*, 50-51.

99 Islam, M. T., Arif, M. R., Hasan, M. T., & Robin, A. H. K. (2023). Anther culture in crop plants: progress and perspectives. *Plant Breeding and Biotechnology, 11*(2), 69–96.

100 Reflinur, Kim, B., Jang, S. M., Chu, S. H., Bordiya, Y., Akter, M. B., & Koh, H. J. (2014). Analysis of segregation distortion and its relationship to hybrid barriers in rice. *Rice, 7*(1), 3.

101 Sun, B., Ding, X., Ye, J., Dai, Y., Cheng, C., Zhou, J., & Chu, H. (2023). Unveiling the Genetic Basis Underlying Rice Anther Culturability via Segregation Distortion Analysis in Doubled Haploid Population. *Genes, 14*(11), 2086.

102 Tripathy, S. K. (2018). Anther culture for double haploid breeding in rice-a way forward. *Rice Genomics and Genetics, 9*.

103 Mayakaduwa, R., & Silva, T. (2023). Haploid induction in indica rice: exploring new opportunities. *Plants, 12*(17), 3118.

104 Sharma, A., Pandey, H., Misra, V., Kumar, R., Vashishth, A., Devadas, V. S., & Chaturvedi, V. D. (2024). CRISPR/Cas9 and anther culture for precision double haploid line production in controlled environments. *Plant Breeding*.

105 Samantaray, S., Ali, J., Nicolas, K. L., Katara, J. L., Verma, R. L., Parameswaran, C., & Bhuyan, S. S. (2021). Doubled haploids in rice improvement: approaches, applications, and future prospects. *In Rice improvement: physiological, molecular breeding and genetic perspectives* (pp. 425–447). Cham: Springer International Publishing.

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Күріш дақылының тозаңдануы: эксперименттік модельден селекциялық тәжірибеге дейін

Дақылдың тозаңдану әдістері гаплоидтар мен дигаплоидтарды жедел алу есебінен селекциялық циклді едәуір қысқартуға мүмкіндік беретін заманауи күріш селекциясының тиімді құралы. Қытай, Жапония, Индия және басқа да елдерде жиналған әлемдік үлкен тәжірибе жоғары өнімді және сапалы жаңа күріш сорттарын шығаруда андрогенез технологиясының өте бағалы екендігін дәлелдейді. Бұл әдістің тиімділігі бірқатар факторларға байланысты, олардың негізгісі — генотип. Тәжірибеде *japonica* түртармағының күріштері каллусогенез және регенерацияға жоғары қабілеттілігімен ерекшеленетіндігі, ал *indica* түртармағы күріштері үшін бұл қасиет төмен болатындығы көрсетілген. Бұндай қиындықтарды шешу үшін будандастыру және протоколда оңтайландырудың әртүрлі әдістері кеңінен қолданылады. Бұл әдіс бірнеше жаңа күріш қатарлары мен сорттары шығарылған Қазақстанда да тиімді болды. Атап айтқанда, өнімділігі жоғары (~5,1 т/га) және құнды, ауылшаруашылық қасиеттері бар «Фатима» сорты еліміздің негізгі күріш өсіретін аймақтарына аудандастырылған. Дақылды тозаңдану арқылы өсіру әдісі Қазақстандағы селекционерлерге бірқатар агрономиялық қасиеттері бойынша бастапқыдан асып түсетін дигаплоидты қатарларды жасауға мүмкіндік берді (мысалы, глютинозды «Виолетта» сортының қатарлары). Күріш дақылының тозаңдануында жаңа генотиптерді және гомозиготалы қатарларды жылдам алуда потенциалы жоғары. Бұл құралды молекулалық тәсілдермен — маркер-ассосацияланған селекциямен (MAS) және CRISPR/Cas9 геномды редакциялау технологияларымен интеграциялауға болады, бұл нақты гендерді сәйкестендіруге және сапалы қасиеттері бар жаңа сорттарды шығару процесін едәуір жеделдетуге мүмкіндік береді.

Кілт сөздер: күріштің тозаңдануы, *in vitro* андрогенез, гаплоидтар, дигаплоидтар, *Oryza sativa* (*indica, japonica*), күріш селекциясы, маркерлік селекция (MAS), CRISPR/Cas9

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Культура пыльников у риса: от экспериментальной модели к селекционной практике

Метод культуры пыльников представляет собой эффективный инструмент современной селекции риса, позволяющий значительно сократить селекционный цикл за счет быстрого получения гаплоидов и дигаплоидов. Огромный мировой опыт, накопленный в Китае, Японии, Индии и других странах, под-

утверждает большую ценность технологии андрогенеза в выведении новых высокопродуктивных и качественных сортов риса. Эффективность данного метода зависит от комплекса факторов, ключевым из которых является генотип. Показано, что подвид риса japonica является значительно более отзывчивым и удобным на практике, тогда как для подвида indica характерна низкая способность к каллусогенезу и регенерации. Для преодоления этих трудностей широко применяется гибридизация и различные способы оптимизации протоколов. Данный метод также зарекомендовал себя и в Казахстане, где получен ряд новых линий и сортов риса. В частности, сорт «Фатима» отличается повышенной урожайностью (~5,1 т/га) и несет ценные хозяйственные признаки, и уже районирован в основных рисосеющих регионах страны. Метод культуры пыльников позволил селекционерам Казахстана вывести дигаплоидные линии, превосходящие исходные по ряду агрономических качеств (например, линии глютинозного сорта «Виолетта»). Обобщая, культура пыльников риса имеет высокий потенциал в ускоренном получении гомозиготных линий и получении новых генотипов. Данный инструмент можно интегрировать с молекулярными подходами — маркер-ассоциированной селекцией (MAS) и технологиями редактирования генома CRISPR/Cas9, что позволит сочетать конкретные гены и значительно ускорить процесс выведения новых сортов с интересующими характеристиками.

Ключевые слова: культура пыльников, андрогенез *in vitro*, гаплоиды, дигаплоиды, *Oryza sativa* (indica, japonica), селекция риса, маркерная селекция (MAS), CRISPR/Cas9

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Asbestos and the anti-asbestos campaign: a review

Assessment of health risks associated with asbestos is based on practical experience, when the asbestos fiber content in the air was artificially increased. Asbestos fibers enter the environment as a result of the erosion of asbestos materials and other human activities not related to the asbestos industry. When specifically searched for, fibers are often detected during autopsies. The results of many studies are more or less biased. When fibers are detected, mesothelioma or lung cancer is sometimes classified as asbestos-related, although the causal relationship remains unproven. Some studies rely on unverified anamnesis on professional or domestic contact with asbestos. Reliable data can be obtained in experiments recording the average lifespan of animals. Different types of asbestos have specific technical characteristics and are used in various fields. For example, asbestos is used in the manufacture of brake pads. Construction materials based on a mixture of cement and asbestos are distinguished by low cost and long service life. Asbestos products are also widely used as fire-resistant materials. On the one hand, the refusal to use asbestos has a positive effect on the environment, but on the other hand, refusing to use it will lead to increased damage from transport and fires, and will increase the cost of construction.

Keywords: asbestos, chrysotile, asbestos-related diseases, mesothelioma, lung cancer, amphiboles.

Introduction

Health risk assessment for asbestos was previously based on high concentrations of asbestos fibers. Risks were assessed using the no-threshold hypothesis, as in the case of radiation hazards, and the effect of low doses of asbestos fibers on the formation of pleural tumors and lung cancer had not been reliably proven [1–3]. There is a view that the harmful effects of asbestos in the workplace and in everyday life effectively ceased in developed countries about 40 years ago, and that modern industrial products and materials do not release any dangerous amounts of fibers [3]. Asbestos fibers are naturally present in the environment, formed as a result of erosion of surface deposits [4-5]. Natural emissions help disperse chrysotile and amphibole asbestos fibers; in areas of natural deposits, asbestos concentrations can significantly exceed those caused by human activity [4, 6]. Any environment, including air, water, soil, and living organisms, can be subject to anthropogenic contamination, including contamination not related to asbestos production and mining. Examples include tunnel digging in asbestos-contaminated soil and other earthworks [7-8]. Research results in Milan showed that asbestos fibers were detected in 35 out of 55 (63.6 %) cases of routine autopsy [9]. During autopsies of deceased individuals who had contact with asbestos, more pieces of lung and pleural tissue are taken for histology, and the examination is performed thoroughly, using special methods. Therefore, asbestos fibers are found more often than in normal autopsies. The detection of fibers alone does not prove either occupational exposure to asbestos or the role of asbestos in the etiology of diseases. It is worth noting that the removal of fibers from the respiratory system is a normal physiological process [9-10].

Compared with other natural sources of pollution, it can be assumed that there is a safe (threshold) concentration of asbestos fibers in various environments, including air. There is experimental data in favor of the existence of thresholds for oncological and other diseases [11]. Apparently, screening and medical examinations have contributed to an increase in the detection of mesothelioma and lung cancer among people who have been exposed to asbestos. Many studies are not sufficiently objective. For example, lung and pleural tumors are sometimes classified as asbestos-related when fibers are detected, although the causal relationship remains unproven. Some studies rely on questionable medical history data on occupational or domestic exposure to asbestos and on interviews with relatives of deceased patients [12].

Experimental

To compile this review, we analyzed literature sources in databases (e-library, Scopus, PubMed, Google Scholar) for the period 1990–2024. Additional information was obtained from dissertations, conference and meeting materials, and specialized literature on the physiology of asbestos-related diseases. The following keywords were used in the search queries: asbestos, lung cancer, mesothelioma, asbestos-related diseases, epidemiological studies.

Review

Malignant pleural mesothelioma (MPM)

The stable incidence of MPM in some developed countries, despite asbestos bans [13–15], is partly due to improvements in diagnostic equipment, the effect of screening, and overdiagnosis due to the unclear definition of MPM as a nosological entity. In addition to natural asbestos, the etiological factors of MPM include artificial and mineral fibers, ionizing radiation, the SV40 virus, chronic lung inflammation such as inflammation, empyema, and tuberculosis, genetic predisposition, and the results of mutagenesis [15–25]. For example, scientists have classified erionite as a stronger carcinogen than asbestos fiber. In general, human activity contributes to the spread of potentially dangerous substances with carcinogenic properties [7, 16]. Even nanomaterials made from traditional components can exhibit carcinogenic properties.

Available publications have found that even viruses, such as the SV40 virus, may contribute to the recent increase in diagnosed cases of mesothelioma [26]. DNA sequences similar to SV40 are regularly found in MPM [27]. After laser microdissection, SV40 was found in MPM cells but not in surrounding cells [26]. When SV40 was introduced, ≥50 % of hamsters developed mesothelial tumors; after injections into the pleural cavity, mesotheliomas occurred in 100 % of hamsters [16, 28]. Thus, an increase in the incidence of MPM was noted in the 1960s, which is associated with the widespread distribution of this virus in 1955–1963, when anti-mumps vaccines contained live SV40 [26]. Antibodies to the SV40 virus were detected in the blood serum of 34 % of MPM patients, compared to 20 % of healthy individuals. The results obtained reliably indicate the involvement of the SV40 virus in the etiology of MPM, as it circulates widely among the populations of different countries [29]. Frequently used invasive procedures, such as bronchoscopy, directly contributed to the spread of the SV40 virus and an increase in additional cases of MPM. Doctors used bronchoscopy and biopsy to diagnose bronchitis associated with asbestos fibers, as well as to identify other dust-related diseases and pneumonia [30–33]. Therefore, even despite the ban on the use of asbestos products, there has been a steady increase in the incidence and mortality rates of MPM [34]. Given the significant presence of carcinogens in the environment, it is expected that most mesothelioma cases will not be diagnosed as asbestos-related [3].

Diagnosing MPM is often difficult. Histologically, MPM can resemble various types of cancer. Tumors can undergo anaplasia and become similar to MPM. The differential diagnosis depends on the subtype of MPM. Diagnosis of the sarcomatoid variant of MPM is particularly difficult; in this case, the usefulness of immunohistochemistry is limited [22, 35]. Reviewing histological archives allows for the identification of misdiagnoses [35-36]. Thus, some studies have shown that the initial diagnosis was confirmed in 67 % of cases, but after review, it was changed to 13 %, with the diagnosis not being accurately established in the remaining cases [37]. Other studies have led to a change in diagnosis for 14 % (of 5,258 cases) of previously diagnosed mesothelioma [16]. According to estimates, about 10 % of MPM cases in the United States were misdiagnosed [36]. The main reason is considered to be the lack of experience of doctors due to the rarity of MPM detection by general practitioners [35, 36]. Whereas in risk groups, MPM is identified by specialized doctors, which leads to a higher detection rate.

Unfortunately, there are no available and reliable biomarkers that could improve the diagnosis of MPM [26]. Immunohistochemical markers such as calretinin, WT1, podoplanin, and HEG1 play a role in diagnosis but do not have sufficient specificity [38]. Previously, mesothelin was considered a reliable marker [39], but its expression is also found in other lung tumors, such as adenocarcinoma [40]. The sensitivity of mesothelin as a marker is insufficient [16, 17, 39, 41]; its expression is often absent in sarcomatoid and epithelioid MPM [35, 42-43]. Osteopontin was considered a promising marker, but the data remain contradictory. Similar to mesothelin, the use of osteopontin and fibulin-3 is limited due to low sensitivity [44]. Information on changes at the molecular level is insufficient [34]. Heterogeneity of chromosomal aberrations in MPM has been noted [24, 45-46]. There are no reliable genetic markers [47-48]. The FISH test can detect the loss of the p16/CDKN2A gene due to 9p21 deletion, which is specific for neoplastic proliferation of

mesothelial cells. However, its sensitivity for MPM is 48–88 % [49]. The authors show an exaggeration of the role of biological immunohistochemical and other molecular markers [39]. The Helsinki Criteria, which were designed to identify a causal link between asbestos and the development of mesothelioma, do not include clear recommendations on the use of biomarkers in screening for MPM diseases [50-51]. Moreover, MPM diseases can show intratumoral variability and subclonality [52]. In other words, markers specific enough for the diagnosis of MPM have not yet been reliably established [50, 53]. Also, malignant tumors diagnosed as MPM did not always differ from other types of cancer. The above explains the increased frequency of MPM detection in risk groups.

Russian science on the dangers of asbestos

Diseases associated with asbestos and its effects were also widespread in the Russian Federation (and the former USSR). Many researchers believed that global bans on the processing and use of asbestos materials were overly strict and that compliance with safety regulations did not lead to contamination and an increase in diseases among the population [30, 54-55]. Thus, there were no studies that proved the risk of developing asbestos-related diseases from low concentrations of asbestos in the environment. No high risks were identified for populations living near various enterprises involved in the production of asbestos fiber. Extensive epidemiological analyses show the presence of safe concentrations of asbestos (asbestos fibers) in the atmosphere [56-57]. It is believed that humans can adapt to certain levels of asbestos fiber concentration [58]. For example, asbestos slate is widely used in construction for roofing. At the same time, the release of asbestos fibers from slate sheets into the environment is very insignificant. It has been established that the average concentration of asbestos fibers in rooms is significantly lower than the maximum permissible level [59]. Asbestos-cement pipes are considered safe for drinking water delivery. The risk of asbestos fibers entering the digestive system has not been proven. It is worth noting that asbestos fibers are almost not separated from the mixture after modification with cement [60-61]. Some studies have assessed the safety of using asbestos-cement pipes for transporting drinking water, and their use has been approved by the Ministry of Health [62]. Consuming water containing 7–10 million fibers per liter does not increase the risk of stomach cancer [63]. Asbestos-containing crushed stone was used in the construction of railway embankments. Its relatively high concentration in the atmosphere has been noted, both at stations and in trains themselves [64]. Asbestos cardboard was widely used, and its carcinogenicity was reduced by aggregation with cellulose fibers [65]. Chrysotile fibers isolated from chrysotile cement have a lower carcinogenic potential than commercial chrysotile. The chrysotile cement industry is considered a source of carcinogenic hazard, but significantly less than asbestos [66]. The toxicity of brake pads containing asbestos fibers has also not been reliably established; no reliable air pollution from such brakes on cars has been recorded, but their effectiveness in road traffic has been noted [67–70]. This fact is related to the fact that materials (cardboard, paper, clothing, gaskets) containing asbestos fibers are still used in various industries [68]. Numerous studies have not found an increase in the incidence of mesothelioma among workers at asbestos plants or among the population living in the vicinity of asbestos plants [71]. An analysis of causal relationships in 3,576 cases of diagnosed mesothelioma showed that asbestos is neither the leading nor the obligatory causal factor [72]. Thus, in Kazakhstan, an analysis of the course of the disease among 69 patients with MPM did not allow a reliable link to be established with the extraction, processing, or use of asbestos [73]. Compliance with Russian MPCs ensures safe working conditions for virtually all workers, i.e., without an increased risk of asbestosis and cancer [63].

Chrysotile and amphibole asbestos

The prevailing opinion is that serpentine asbestos (chrysotile) is less toxic than amphibole asbestos (actinolite, tremolite, amosite, crocidolite, anthophyllite, etc.), but there are contradictions between the data from epidemiological and experimental studies. In Russia, almost exclusively chrysotile is produced. Some experts believed that the opinion about the danger of certain forms of asbestos was not sufficiently substantiated [74]. Thus, the cytotoxic, carcinogenic, mutagenic, and fibrogenic effects of chrysotile have been reliably confirmed by a number of epidemiological studies, as well as in experiments [75–77]. Comparative experiments have determined that anthophyllite is less dangerous than chrysotile in terms of its fibrogenic effect [78]. In laboratory experiments, chrysotile was sufficiently toxic to cause a noticeable granulomatous tissue reaction [79]. However, its carcinogenic effect did not differ significantly from other types of asbestos [80]. The studies comment: “After short-term exposure, longer chrysotile fibers are quickly removed from the lungs” [81]. Since chrysotile fibers can migrate from lung tissue to the pleura [82–87], it is very difficult to assess biopersistence solely by counting asbestos fibers in the lungs. Bernstein’s research protocol [81] is the reason for the very long half-life of the fibers. Therefore, the carcinogenicity of chrysotile is

considered insignificant. However, some of Bernstein's conclusions contradict the results obtained by independent researchers. Perhaps these results are related to aggressive sample preparation when determining asbestos fibers in the lungs [88]. The decomposition of asbestos by acids does not prove its solubility in tissues in vivo. With references to the named author, unfounded statements are made: "It has been shown that chrysotile is rapidly removed from the lungs of experimental animals after inhalation"; "chrysotile, which rapidly decomposes in the lungs, behaves more like non-fibrous mineral dust" [89].

Experiments were conducted on the dissolution of asbestos fibers in Gamble's solution, which mimics the interstitial fluid of the lungs. Solubility ranged from a few nanograms of dissolved silicon per square centimeter of fiber surface (chrysotile and crocidolite) to thousands $^{of\ ng/cm^2}$ (glass fiber). However, aramid and carbon fibers proved to be practically insoluble [90]. Experiments with Gamble's solution showed that a relatively large amount of magnesium dissolves from chrysotile. Silicates are based on silicon and oxygen atoms in Si-O-Si chains. The strength of the fibers is mainly determined by the bonds between these atoms. Electrostatic forces act between the chains due to negatively charged oxygen atoms bound to silicon atoms and cations, including magnesium [82, 91–93]. The leaching of magnesium from the surface of the fibers can contribute to their longitudinal splitting. As a result, the total number of thin asbestos fibers can increase significantly [82, 83, 87, 92–97], causing an increase in the carcinogenic effect. The authors suggested that an increase in fiber thickness leads to an increase in the carcinogenic effect due to better penetration into human and animal tissues [97]. Further research in this area is needed.

Thus, the rapid removal of chrysotile from lung tissue can be explained by the breakdown of fibers into thin fragments that are difficult to identify. Asbestos fibers are usually found in the pleura postmortem, with chrysotile being the predominant fiber in the pleura and pleural plaques [85, 86, 98, 99]. The idea of fiber migration from the lungs to the pleura is consistent with the proven fact that the primary focus of mesothelioma in individuals who have been exposed to asbestos is more often located in the parietal pleura than in the visceral pleura [100]. A number of studies have confirmed the biopersistence of chrysotile in the human lungs [101]. However, it cannot be ruled out that chrysotile dissolves more quickly in the acidic environment of lysosomes. In experiments on rats, chrysotile caused inflammation in a relatively short period of time, followed by malignant tumors, while crocidolite had a carcinogenic effect at a later stage [102].

It has been noted that the incidence of mesothelioma increases significantly when exposed to pure chrysotile [103-104]. Thus, the significantly higher incidence of mesothelioma among workers who had contact with amphiboles was explained by higher doses of this substance [105]. As mentioned above, there are discrepancies between the results of animal experiments and epidemiological data. It has been noted that the evidence for differences between chrysotile and amphiboles in lung cancer is "weak at best" [106]. Some experiments have demonstrated virtually identical carcinogenic activity of amphiboles and chrysotile in relation to mesothelioma [93, 107–109] and lung cancer [110-111]. However, some studies show a higher level of carcinogenic effect of chrysotile compared to amphibole. Thus, it was noted: "No evidence of lower carcinogenicity or less severe asbestosis was found in groups exposed to chrysotile compared to groups exposed to amphiboles" [109]. In experiments on laboratory animals (rats), chrysotile caused a greater number of tumors and pulmonary fibrosis compared to amphibole. This is explained by the higher concentration of fibers longer than 20 μm in the chrysotile used in the experiment [112]. An increase in chrysotile concentration contributed to the occurrence of chromosomal breaks, which led to pre-tumor transformation of cells in vitro [107, 113].

In humans, the difference in the risk of developing lung cancer between chrysotile and amphiboles (amosite and crocidolite) has been determined to be between 1:10 and 1:50. For mesothelioma, the risk ratio from exposure to these types of asbestos was estimated to be 1:100:500, respectively [2]. The latter risk ratio was described in sources [37, 114]. In a later report, this risk was estimated at a ratio of 1:5:10 [115]. The authors noted that in experiments with laboratory animals, all types of asbestos provoke almost the same number of lung tumors, which shows a contradiction between epidemiological and experimental studies. The following explanation was proposed for this situation: "In humans, chrysotile fibers (excreted over months) may have less effect than amphibole fibers (excreted over years)" [2]. However, no different mechanisms for the removal of fibers from the tissues of the respiratory system have been proposed. That is, a decrease in the concentration of chrysotile in the lungs may be caused by the breakdown of asbestos fibers and their movement into the pleural tissue.

The toxicity of asbestos and other types of fibers largely depends on the three "Ds"—Dose, Dimensions, Durability [18, 116–118]. If different types of asbestos fibers have the same biopersistence indicators, then the varying degrees of carcinogenicity depend on the thickness and length of the fibers [119]. Thus,

long chrysotile fibers showed significantly higher toxicity, as they are less readily absorbed by macrophages [120–121]. According to another study, short and thin chrysotile fibers predominated in MPM in the lungs and pleura [122]. It has been noted that tremolite impurities in chrysotile products contribute to an increased carcinogenic effect [123]. In an epidemiological study, the difference in the risk of MPM from pure chrysotile and its mixtures with amphiboles was found to be insignificant [124].

The toxicity of different types of asbestos was compared in a meta-analysis of 19 epidemiological studies, which assessed the impact of study quality on the dose-response relationship for lung cancer. The difference between amphiboles and chrysotile was significantly lower when the meta-analysis was limited to high-quality studies [114, 125]. After standardization for quality, the difference between the two types of fibers was not significant [114, 126].

The overall estimates of the risk of lung cancer were higher after exposure to amphiboles — 1.74 (95 % confidence interval 1.18-2.57), and slightly lower after exposure to chrysotile — 0.99 (0.78-1.25) [127]. The significant differences between the results of high- and low-quality studies indicate that the latter lack objectivity. As mentioned above, the prevailing opinion is that chrysotile is less toxic than amphiboles. This difference should be quantitatively assessed in independent studies.

Discussion

Asbestos bans were partly based on studies that were influenced by economic interests. When determining the criteria for including studies in reviews and meta-analyses, their quality and possible systematic errors should be taken into account. Objective information can be obtained from laboratory animal experiments with determination of average life expectancy. That is, it is desirable to use large animals; the best results for humans can be obtained by testing asbestos on primates [128]. Experiments involving the inhalation of fibers in doses comparable to those in the asbestos industry are ethically acceptable, as they can be carried out without the use of invasive procedures. Experiments and studies using “concentrations many times higher than those found in the workplace” [129] have limited reliability. For example, replacing asbestos with artificial fibers is unlikely to eliminate the risks to health and the development of lung diseases [18, 19, 130-131]. Thus, at present, carcinogenic effects are already being detected in materials used as substitutes for asbestos fiber, such as carbon nanotubes. Studies show that asbestos fibers and carbon nanotubes have toxic effects through the same mechanisms, in particular, chronic activation of macrophages, leading to inflammation [132]. Nanoparticles can cause structural changes in membrane proteins and activate the synthesis of inflammatory mediators, disrupting normal cellular metabolism mechanisms [133]. An experiment has demonstrated the carcinogenic effect of nanotubes [20, 134]. Carbon nanotubes are biostable, and some of their varieties have been classified as possible human carcinogens [135].

The extraction and use of asbestos is prohibited in a number of countries, while others continue to produce and export it [136]. Chrysotile products traded internationally contain impurities of varying amounts of amphiboles [137]. Different types of asbestos have their own technical advantages and preferred areas of application. Amphiboles (crocidolite, anthophyllite) are acid-resistant, thermally stable, and durable [138].

Conclusion

Asbestos is an inexpensive material and an effective reinforcing fiber. Asbestos cement structures are durable and fire resistant. Asbestos-based products are highly durable and safe, and incorporating them into other products increases their reinforcing properties. It can be confidently assumed that the refusal to use asbestos-containing materials will increase the damage and number of victims of road accidents, fires, and armed conflicts. The independence of scientific research from economic interests is of great importance. However, the mechanism of development of many respiratory diseases from asbestos exposure has not been reliably established, which requires further research.

Conflict of Interest

Author declares no conflict of interest.

Author contribution

The manuscript was written through contributions of author. The author has given approval to the final version of the manuscript: **Jargin S.V.** — conceptualization, investigation, data collection, draft writing.

References

1 Jargin S.V. Asbestos-related Cancer: Exaggerated Risk Perception / S.V. Jargen // Cancer Screening and Prevention. — 2023. — Vol. 2(1). — P. 51–57. DOI: 10.14218/CSP.2022.00028

2 Hodgson J.T. The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure / J.T. Hodgson, A. Darnton // Annals of Occupational Hygiene. — 2000. — Vol. 44. — P. 565–601.

3 Paustenbach D. A critical review of the 2020 EPA risk assessment for chrysotile and its many shortcomings / D. Paustenbach, D. Brew, S. Ligas, J. Heywood // Critical Reviews in Toxicology. — 2021. — Vol. 51(6). — P. 509–539. DOI: 10.1080/10408444.2021.1968337

4 Noonan C.W. Environmental asbestos exposure and risk of mesothelioma / C.W. Noonan // Annals of Translational Medicine. — 2017. — Vol. 5(11). — P. 234. DOI: 10.21037/atm.2017.03.74

5 Peña-Castro M. A critical review of asbestos concentrations in water and air, according to exposure sources / M. Peña-Castro, M. Montero-Acosta, M. Saba // Heliyon. — 2023. — Vol. 9(5). — e15730. DOI: 10.1016/j.heliyon.2023.e15730

6 Ilgren E. Further studies of Bolivian crocidolite — Part IV: Fibre width, fibre drift and their relation to mesothelioma induction: Preliminary findings / E. Ilgren, D.R. Van Orden, R.J. Lee, Y.M. Kamiya, J.A. Hoskins // Epidemiology Biostatistics and Public Health. — 2015. — Vol. 12. — e11167-1. DOI: 10.2427/11167

7 Berry T.A. Asbestos and other hazardous fibrous minerals: potential exposure pathways and associated health risks / T.A. Berry, E. Belluso, R. Vigliaturo, R. Gieré, E.A. Emmett, J.R. Testa, G. Steinhorn, S.L. Wallis // International Journal of Environmental Research and Public Health. — 2022. — Vol. 19(7). — P. 4031. DOI: 10.3390/ijerph19074031

8 Malinconico S. Asbestos in soil and water: A review of analytical techniques and methods / S. Malinconico, F. Paglietti, S. Serranti, G. Bonifazi, I. Lonigro // Journal of Hazardous Materials. — 2022. — Vol. 436. — 129083. DOI: 10.1016/j.jhazmat.2022.129083

9 Casali M. Asbestos lung burden in necroscopic samples from the general population of Milan, Italy / M. Casali, M. Carugno, A. Cattaneo, D. Consonni, C. Mensi, D.M. Cavallo, A. Somigliana, A.C. Pesatori // Annals of Occupational Hygiene. — 2015. — Vol. 59. — P. 909–921. DOI: 10.1093/annhyg/mev028

10 Bayram M. Environmental exposure to asbestos: from geology to mesothelioma / M. Bayram, N.D. Bakan // Current Opinion in Pulmonary Medicine. — 2014. — Vol. 20. — P. 301–307. DOI: 10.1097/MCP.0000000000000053

11 Goodman J.E. Challenges in defining thresholds for health effects: some considerations for asbestos and silica / J.E. Goodman, L.R. Rhomberg, S.M. Cohen, K.A. Mundt, B. Case, I. Burstyn, M.J. Becich, G. Gibbs // Front Epidemiol. — 2025. — Vol. 5. — 1557023. DOI: 10.3389/fepid.2025.1557023

12 Yang H. Mesothelioma epidemiology, carcinogenesis, and pathogenesis / H. Yang, J.R. Testa, M. Carbone // Current Treatment Options in Oncology. — 2008. — Vol. 9. — P. 147–157. DOI: 10.1007/s11864-008-0067-z

13 Kraus T. Mesotheliome — 30 Jahre nach dem Asbestverbot in Deutschland / T. Kraus, D. Jonigk // Pathologie (Heidelb). — 2024. — Vol. 45(5). — P. 305–308. DOI: 10.1007/s00292-024-01350-5

14 Nel A.E. The Interplay Between the Immune System, Tumor Suppressor Genes, and Immune Senescence in Mesothelioma Development and Response to Immunotherapy / A.E. Nel, E.N. Pavlisko, V.L. Roggli // J Thorac Oncol. — 2024. — Vol. 19(4). — P. 551–564. DOI: 10.1016/j.jtho.2023.11.017

15 Nash A. Genomic landscape of pleural mesothelioma and therapeutic aftermaths / A. Nash, J. Creaney // Curr Oncol Rep. — 2023. — Vol. 25(12). — P. 1515–1522. DOI: 10.1007/s11912-023-01479-1

16 Carbone M. Mesothelioma: Scientific clues for prevention, diagnosis, and therapy / M. Carbone, P.S. Adusumilli, H.R. Alexander Jr, P. Baas, F. Bardelli, A. Bononi, R. Bueno, E. Felley-Bosco, F. Galateau-Salle, D. Jablons, A.S. Mansfield, M. Minaai, M. de Perrot, P. Pesavento, V. Rusch, D.T. Severson, E. Taioli, A. Tsao, G. Woodard, H. Yang, M.G. Zauderer, H.I. Pass // CA: a Cancer Journal For Clinicians. — 2019. — Vol. 69(5). — P. 402–429. DOI: 10.3322/caac.21572

17 Dipper A. Ancillary diagnostic investigations in malignant pleural mesothelioma / A. Dipper, N. Maskell, A. Bibby // Cancers (Basel). — 2021. — Vol. 13(13). — 3291. DOI: 10.3390/cancers13133291

18 Donaldson K. Pulmonary toxicity of carbon nanotubes and asbestos — similarities and differences / K. Donaldson, C.A. Poland, F.A. Murphy, M. MacFarlane, T. Chernova, A. Schinwald // Advanced Drug Delivery Reviews. — 2013. — Vol. 65. — P. 2078–2086. DOI: 10.1016/j.addr.2013.07.014

19 Greim H. Perspectives on refractory ceramic fiber (RCF) carcinogenicity: comparisons with other fibers / H. Greim, M.J. Utell, L.D. Maxim, R. Niebo // Inhalation Toxicology. — 2014. — Vol. 26. — P. 789–810. DOI: 10.3109/08958378.2014.953276

20 Janosikova M. Current causes of mesothelioma: how has the asbestos ban changed the perspective? / M. Janosikova, M. Nakladalova, L. Stepanek // Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. — 2023. — Vol. 167(2). — P. 99–108. DOI: 10.5507/bp.2023.008

21 Jasani B. Mesothelioma not associated with asbestos exposure / B. Jasani, A. Gibbs // Archives of Pathology and Laboratory Medicine. — 2012. — Vol. 136. — P. 262–267. DOI: 10.5858/arpa.2011-0039-RA

22 Panou V. The established and future biomarkers of malignant pleural mesothelioma / V. Panou, M. Vyberg, U.M. Weinreich, C. Meristoudis, U.G. Falkmer, O.D. Røe // Cancer Treatment Reviews. — 2015. — Vol. 41. — P. 486–495. DOI: 10.1016/j.ctrv.2015.05.001

23 Røe O.D. Malignant pleural mesothelioma: history, controversy and future of a manmade epidemic / O.D. Røe, G.M. Stella // European Respiratory Review. — 2015. — Vol. 24. — P. 115–131. DOI: 10.1183/09059180.00007014

24 Røe O.D. Genome-wide profile of pleural mesothelioma versus parietal and visceral pleura: the emerging gene portrait of the mesothelioma phenotype / O.D. Røe, E. Anderssen, E. Helge, C.H. Pettersen, K.S. Olsen, H. Sandeck, R. Haaverstad, S. Lundgren, E. Larsson // PLoS One. — 2009. — e6554. DOI: 10.1371/journal.pone.0006554

25 Rossini M. New perspectives on diagnosis and therapy of malignant pleural mesothelioma / M. Rossini, P. Rizzo, I. Bononi, A. Clementz, R. Ferrari, F. Martini, M.G. Tognon // Frontiers of Oncology. — 2018. — Vol. 8. — P. 91. DOI: 10.3389/fonc.2018.00091

26 Carbone M. SV40 and human mesothelioma / M. Carbone, A. Gazdar, J.S. Butel // Translational Lung Cancer Research. — 2020. — Vol. 9(Suppl 1). — S47–S59. DOI: 10.21037/tlcr.2020.02.03

27 Testa J.R. A multi-institutional study confirms the presence and expression of simian virus 40 in human malignant mesotheliomas / J.R. Testa, M. Carbone, A. Hirvonen, K. Khalili, B. Krynska, K. Linnainmaa, F.D. Pooley, P. Rizzo, V. Rusch, G.H. Xiao // Cancer Research. — 1998. — Vol. 58(20). — P. 4505–4509.

28 Cicala C. SV40 induces mesotheliomas in hamsters / C. Cicala, F. Pompetti, M. Carbone // American Journal of Pathology. — 1993. — Vol. 142. — P. 1524–1533.

29 Mazzoni E. Sera from patients with malignant pleural mesothelioma tested positive for IgG antibodies against SV40 large t antigen: the viral oncoprotein / E. Mazzoni, I. Bononi, J.C. Rotondo, C. Mazziotta, R. Libener, R. Guaschino, R. Gafà, G. Lanza, F. Martini, M. Tognon // J. Oncol. — 2022. — Vol. 2022. — 7249912. DOI: 10.1155/2022/7249912

30 Еловская Л.Т. Антиасбестовая кампания и конференция по проблеме «Асбест и здоровье» / Л.Т. Еловская // Медицина труда и промышленная экология. — 1997. — № 9. — С. 16–21.

31 Казанцев В.А. Применение бронхологической санации для лечения больных внебольничными пневмониями / В.А. Казанцев // 14-й Нац. конгресс: сб. — М.; Рос. респираторное о-во; 2004. — С. 361.

32 Лихачева Е.И. Клинические особенности заболеваний легких от воздействия пыли хризотил-асбеста / Е.И. Лихачева, А.Л. Ярина, Е.Р. Вагина, М.С. Климина, Т.И. Обухова, Т.А. Довголюк, С.В. Кашанский // Медицина труда и промышленная экология. — 2000. — № 11. — С. 30–33.

33 Милишникова В.В. Эндоскопическая и морфологическая характеристика бронхов и легких при асбестозе и пылевом бронхите у работников асботекстильной промышленности / В.В. Милишникова, И.Ю. Лощилов, Е.В. Гладкова, А.О. Аксенова, Л.А. Туркина // Гигиена труда и профессиональные заболевания. — 1990. — № 7. — С. 19–22.

34 Lorenzini E, Ciarrocchi A, Torricelli F. Molecular fingerprints of malignant pleural mesothelioma: not just a matter of genetic alterations / E. Lorenzini, A. Ciarrocchi, F. Torricelli // Journal of Clinical Medicine. — 2021. — Vol. 10(11). — P. 2470. DOI: 10.3390/jcm10112470

35 Carbone M. Mesothelioma: recent highlights / M. Carbone, H. Yang // Annals of Translational Medicine. — 2017. — Vol. 5(11). — P. 238. DOI: 10.21037/atm.2017.04.29

36 Chen Z. Diagnostic and prognostic biomarkers for malignant mesothelioma: an update / Z. Chen, G. Gaudino, H.I. Pass, M. Carbone, H. Yang // Translational Lung Cancer Research. — 2017. — Vol. 6. — P. 259–269. DOI: 10.21037/tlcr.2017.05.06

37 Goldberg M. The French national mesothelioma surveillance program / M. Goldberg, E. Imbernon, P. Rolland, A. Gilg Soit Ilg, M. Savès, A. de Quillacq, C. Frenay, S. Chamming's, P. Arveux, C. Boutin, G. Launoy, J.C. Pairon, P. Astoul, F. Galateau-Sallé, P. Brochard // Occupational and Environmental Medicine. — 2006. — Vol. 63. — P. 390–395. DOI: 10.1136/oem.2005.023200

38 Lucà S. Diagnostic challenges in the pathological approach to pleural mesothelioma / S. Lucà, G. Pignata, A. Cioce, C. Salzillo, R. De Cecio, G. Ferrara, C.M. Della Corte, F. Morgillo, A. Fiorelli, M. Montella, R. Franco // Cancers (Basel). — 2025. — Vol. 17(3). — P. 481. DOI: 10.3390/cancers17030481

39 Creaney J. Discovery of new biomarkers for malignant mesothelioma / J. Creaney, I.M. Dick, B.W. Robinson // Curr Pulmonology Reports. — 2015. — Vol. 4. — P. 15–21. DOI: 10.1007/s13665-015-0106-8

40 Ho M. Mesothelin expression in human lung cancer / M. Ho, T.K. Bera, M.C. Willingham, M. Onda, R. Hassan, D. FitzGerald, I. Pastan // Clinical Cancer Research. — 2007. — Vol. 13(5). — P. 1571–1575. DOI: 10.1158/1078-0432.CCR-06-2161

41 Bibby A.C. Malignant pleural mesothelioma: an update on investigation, diagnosis and treatment / A.C. Bibby, S. Tsim, N. Kanellakis, H. Ball, D.C. Talbot, K.G. Blyth, N.A. Maskell, I. Psallidas // European Respiratory Review. — 2016. — Vol. 25. — P. 472–486. DOI: 10.1183/16000617.0063-2016

42 Grigoriu B.D. Clinical utility of diagnostic markers for malignant pleural mesothelioma / B.D. Grigoriu, C. Grigoriu, B. Chahine, T. Gey, A. Scherpereel // Monaldi Archive of Chest Diseases. — 2009. — Vol. 71(1). — P. 31–38. DOI: 10.4081/monaldi.2009.374

43 Pantazopoulos I. Effectiveness of mesothelin family proteins and osteopontin for malignant mesothelioma / I. Pantazopoulos, P. Boura, T. Xanthos, K. Syrigos // European Respiratory Journal. — 2013. — Vol. 41(3). — P. 706–715. DOI: 10.1183/09031936.00226111

44 Harris E.J.A. Diagnosis of asbestos-related lung diseases / E.J.A. Harris, A. Musk, N. de Klerk, A. Reid, P. Franklin, F.J.H. Brims // Expert Review of Respiratory Medicine. — 2019. — Vol. 13(3). — P. 241–249. DOI: 10.1080/17476348.2019.1568875

45 Lindholm P.M. Gene copy number analysis in malignant pleural mesothelioma using oligonucleotide array CGH / P.M. Lindholm, K. Salmenkivi, H. Vauhkonen, A.G. Nicholson, S. Anttila, V.L. Kinnula, S. Knuutila // Cytogenetic and Genome Research. — 2007. — Vol. 119. — P. 46–52. DOI: 10.1159/000109618

46 Musti M. Cytogenetic and molecular genetic changes in malignant mesothelioma / M. Musti, E. Kettunen, S. Dragonieri, P. Lindholm, D. Cavone, G. Serio, S. Knuutila // Cancer Genetics and Cytogenetics. — 2006. — Vol. 170. — P. 9–15. DOI: 10.1016/j.cancergencyto.2006.04.011

47 Cersosimo F. Mesothelioma malignancy and the microenvironment: molecular mechanisms / F. Cersosimo, M. Barbarino, S. Lonardi, W. Vermi, A. Giordano, C. Bellan, E. Giurisato // Cancers (Basel). — 2021. — Vol. 13(22). — 5664. DOI: 10.3390/cancers13225664

48 Vandenhoeck J. DNA methylation as a diagnostic biomarker for malignant mesothelioma: a systematic review and meta-analysis / J. Vandenhoeck, J.P. van Meerbeeck, E. Fransen, J. Raskin, G. Van Camp, K. Op de Beeck, K. Lamote // Journal of Thoracic Oncology. — 2021. — Vol. 16(9). — P. 1461–1478. DOI: 10.1016/j.jtho.2021.05.015

49 Zahiu T. Molecular Insights into Pleural Mesothelioma: Unveiling Pathogenic Mechanisms and Therapeutic Opportunities / T. Zahiu, C.M. Mihu, B.A. Bosca, M. Mărginean, L.P. Mocan, R.A. Ștefan, R.T. Sufleţel, C. Mihu, C.S. Melincovici // Diagnostics (Basel). — 2025. — Vol. 15(11). — 1323. DOI: 10.3390/diagnostics15111323

50 Ferrari L. Circulating epigenetic biomarkers in malignant pleural mesothelioma: state of the art and critical evaluation / L. Ferrari, M. Carugno, C. Mensi, A.C. Pesatori // Frontiers of Oncology. — 2020. — Vol. 10. — 445. DOI: 10.3389/fonc.2020.00445

51 Wolff H. Asbestos, asbestosis, and cancer, the Helsinki criteria for diagnosis and attribution 2014: recommendations / H. Wolff, T. Vehmas, P. Oksa, J. Rantanen, H. Vainio // Scandinavian Journal of Work and Environmental Health. — 2015. — Vol. 41(1). — P. 5–15. DOI: 10.5271/sjweh.3462

52 Rossi G. When the diagnosis of mesothelioma challenges textbooks and guidelines / G. Rossi, F. Davoli, V. Poletti, A. Cavazza, F. Lococo // Journal of Clinical Medicine. — 2021. — Vol. 10(11). — P. 2434. DOI: 10.3390/jcm10112434

53 Schillebeeckx E. Clinical utility of diagnostic biomarkers in malignant pleural mesothelioma: a systematic review and meta-analysis / E. Schillebeeckx, J.P. van Meerbeeck, K. Lamote // European Respiratory Review. — 2021. — Vol. 30(162). — P. 210057. DOI: 10.1183/16000617.0057-2021

54 Измеров Н.Ф. Нормативное обеспечение контролируемого использования асбестосодержащих материалов в строительстве / Н.Ф. Измеров, Е.В. Ковалевский // Медицина труда и промышленная экология. — 2004. — № 5. — С. 5–12.

55 Нейман С.М. О безопасности асбестоцементных материалов и изделий / С.М. Нейман, А.И. Везенцев, С.В. Кашанский. — М.: Стройматериалы, 2006. — 120 с.

56 Коган Ф.М. Влияние низкой концентрации асбестосодержащей пыли / Ф.М. Коган, С.В. Кашанский, Е.Г. Плотко, С.А. Берзин, Г.Б. Богданов // Медицина труда и промышленная экология. — 1993. — № 5-6. — С. 6–10.

57 Штоль А.В. Загрязнение атмосферного воздуха асбестосодержащей пылью и здоровье детского населения / А.В. Штоль, Е.Г. Плотко, К.П. Селянкина // Медицина труда и промышленная экология. — 2000. — № 11. — С. 10–13.

58 Цурикова Г.В. Биодемографические параметры как индикаторы генетической адаптации к вредным профессиональным факторам на примере асбеста / Г.В. Цурикова, В.А. Спицын, Е.В. Гладкова, О.П. Минаева // Гигиена труда и профессиональные заболевания. — 1992. — № 6. — С. 28–30.

59 Кашанский С.В. Современные проблемы асбеста и перспективные направления исследований / С.В. Кашанский, С.Г. Домнин, Е.Г. Плотко, С.В. Кузьмин, С.В. Селянкина, Е.И. Лихачева // Медицина труда и промышленная экология. — 2004. — № 9. — С. 16–19.

60 Красовский Г.Н. Асбест и качество питьевой воды / Г.Н. Красовский, Н.А. Егорова // Гигиена и санитария. — 1985. — № 3. — С. 64–67.

61 Красовский Г.Н. Асбест в питьевой воде (обзор) / Г.Н. Красовский, Е.А. Можаев // Гигиена и санитария. — 1993. — № 6. — С. 20–22.

62 Репина Ж.В. Хризотилцементные строительные материалы. Области использования / Ж.В. Репина, Н.А. Чемякина, Е.Г. Тарская-Лаптева. — Екатеринбург: АМБ, 2009. — 156 с.

63 Коган Ф.М. Основные результаты исследований по проблеме «Асбест-Здоровье» / Ф.М. Коган; подгот. С.В. Кашанским и др. // В кн.: // Профилактика асбестообусловленных заболеваний: Сб. публ. — Асбест: Асбестовая ассоциация, 2002. — С. 7–9.

64 Капцов В.А. Железнодорожное использование асбестосодержащего щебня: эколого-гигиенические аспекты / В.А. Капцов, С.В. Кашанский, С.Г. Домнин, Т.С. Тихова, Е.В. Трофимова, Т.А. Новоселова, Г.Б. Богданов // Гигиена и санитария. — 2003. — № 5. — С. 11–15.

65 Кашанский С.В. Опасность развития рака легких при производстве асбестовых панелей / С.В. Кашанский, Ф.М. Коган // Медицина труда и промышленная экология. — 1995. — № 5. — С. 19–22.

66 Пылев Л.Н. Хризотилцементное производство — источник канцерогенной опасности для человека? / Л.Н. Пылев, О.В. Смирнова, А.И. Везенцев // Токсикологический вестник. — 2011. — № 4. — С. 46–50.

67 Яценко А.С. О взаимосвязи между биологической агрессивностью и некоторыми физико-химическими свойствами промышленной пыли, возникающей при производстве и применении фрикционных изделий / А.С. Яценко, Ф.М. Коган, А.С. Фомина, В.А. Зыкова, О.В. Никитина, Н.Н. Ванчугова, Г.Б. Богданов, С.И. Алиамовский, Л.А. Переляева // Медицина труда и промышленная экология. — 1994. — № 12. — С. 29–33.

68 Ковалевский Е.В. Гигиеническая оценка применения асбестосодержащих фрикционных изделий / Е.В. Ковалевский // Медицина труда и промышленная экология. — 2009. — № 7. — С. 1–6.

69 Яценко А.С. Профессиональная заболеваемость и смертность от злокачественных новообразований среди лиц, имеющих профессиональный контакт с асбестовой пылью / А.С. Яценко, Ф.М. Коган // Гигиена труда и профессиональные заболевания. — 1990. — № 2. — С. 10–12.

70 Яценко А.С. Сравнительное исследование фибриногенной активности пыли при производстве асбестоформованных деталей / А.С. Яценко, Ф.М. Коган, Л.Н. Эльничных, И.И. Ремизова // Гигиена и санитария. — 1991. — № 8. — С. 27–29.

71 Измеров Н.Ф. Хризотил-асбест в России: некоторые результаты и перспективные направления исследований / Н.Ф. Измеров, Л.Т. Еловская, В.В. Милишникова, Т.Б. Бурмистрова, Е.В. Ковалевский // Медицина труда и промышленная экология. — 1998. — № 10. — С. 1–7.

72 Кашанский С.В. Мезотелиома в России: системный обзор 3576 опубликованных случаев с позиции медицины труда / С.В. Кашанский // Медицина труда и промышленная экология. — 2008. — № 3. — С. 15–21.

73 Кашанский С.В. Мезотелиома в Республике Казахстан: обзор / С.В. Кашанский, Б.А. Жетписбаев, О.З. Ильдербаев, О.Т. Ерменбай // Гигиена и санитария. — 2008. — № 5. — С. 13–17.

74 Коган Ф.М. Современные представления о безопасности асбеста / Ф.М. Коган. — Екатеринбург: Арго, 1995. — 95 с.

75 Пылев Л.Н. Канцерогенная активность асбестоцементной пыли / Л.Н. Пылев, Ф.М. Коган, Т.Ф. Кулагина // Гигиена труда и профессиональные заболевания. — 1988. — № 7. — С. 55–57.

76 Пылев Л.Н. Экспериментальное обоснование канцерогенной опасности асбестоцементной промышленности и ее продукции / Л.Н. Пылев, О.В. Смирнова, Л.А. Васильева, С.А. Хрусталев, А.И. Везенцев, Е.А. Гудкова, Л.Н. Наумова // Гигиена и санитария. — 2010. — № 6. — С. 61–65.

77 Троицкая Н.А. Сравнительное исследование цитотоксичности пыли углеродных волокон и других волокнистых материалов / Н.А. Троицкая // Гигиена и санитария. — 1993. — № 3. — С. 28–30.

78 Коган Ф.М. К вопросу о нормировании асбестсодержащих пылей в воздухе рабочих помещений / Ф.М. Коган; подгот. С.В. Кашанским и др. // В кн.: Профилактика асбестообусловленных заболеваний: Сб. публ. — Асбест: Асбестовая ассоциация, 2002. — С. 57–63.

79 Кашанский С.В. Сравнительная оценка фиброгенеза и токсичности асбестосодержащих теплоизоляционных материалов / С.В. Кашанский, Ф.М. Коган, Л.Г. Малышева, В.А. Зыкова // Медицина труда и промышленная экология. — 1994. — № 1. — С. 17–21.

80 Пылев Л.Н. Роль модифицирующих факторов в канцерогенном действии асбеста и асбестосодержащих пылей / Л.Н. Пылев // Экспериментальная онкология. — 1987. — № 9(5). — С. 14–17.

81 Bernstein D.M. The health risk of chrysotile asbestos / D.M. Bernstein // Current Opinion in Pulmonary Medicine. — 2014. — Vol. 20. — P. 366–370. DOI: 10.1097/MCP.0000000000000064

82 Coin P.G. Persistence of long, thin chrysotile asbestos fibers in the lungs of rats / P.G. Coin, V.L. Roggli, A.R. Brody // Environ Health Perspective. — 1994. — Vol. 102. — P. 197–199. DOI: 10.1289/ehp.94102s5197

83 Kohyama N. Analysis of asbestos fibers in lung parenchyma, pleural plaques, and mesothelioma tissues of North American insulation workers / N. Kohyama, Y. Suzuki // Annals of the New York Academy of Sciences. — 1991. — Vol. 643. — P. 27–52. DOI: 10.1111/j.1749-6632.1991.tb24442.x

84 Nicholson W.J. Comparative dose-response relationships of asbestos fiber types: magnitudes and uncertainties / W.J. Nicholson // Annals of the New York Academy of Sciences. — 1991. — Vol. 643. — P. 74–84. DOI: 10.1111/j.1749-6632.1991.tb24446.x

85 Sebastien P. Asbestos retention in human respiratory tissues: comparative measurements in lung parenchyma and in parietal pleura / P. Sebastien, X. Janson, A. Gaudichet, A. Hirsch, J. Bignon // IARC Scientific Publications. — 1980. — Vol. 30. — P. 237–246.

86 Stayner L.T. Occupational exposure to chrysotile asbestos and cancer risk: a review of the amphibole hypothesis / L.T. Stayner, D.A. Dankovic, R.A. Lemen // American Journal of Public Health. — 1996. — Vol. 86. — P. 179–186. DOI: 10.2105/ajph.86.2.179

87 Suzuki Y. Asbestos fibers contributing to the induction of human malignant mesothelioma / Y. Suzuki, S.R. Yuen // Annals of the New York Academy of Sciences. — 2002. — Vol. 982. — P. 160–176. DOI: 10.1111/j.1749-6632.2002.tb04931.x.24

88 Pezerat H. Chrysotile biopersistence: the misuse of biased studies / H. Pezerat // International Journal of Occupational and Environmental Health. — 2009. — Vol. 15. — P. 102–106. DOI: 10.1179/107735209799449770

89 Койгельдинова Ш.С. Современный взгляд на проблему профессиональных заболеваний легких от воздействия хризотил-асбеста / Ш.С. Койгельдинова, С.А. Ибраев, Г.О. Жузбаева, А.К. Касымова // Вестник Карагандинского университета. Серия «Биология. Медицина. География». — 2015. — № 79(3). — С. 122–131.

90 Larsen G. Experimental data on in vitro fiber solubility / G. Larsen // IARC Scientific Publications. — 1989. — Vol. 90. — P. 134–139.

91 Федосеев А.Д. Волокнистые силикаты. Природные и синтетические асбесты / А.Д. Федосеев, Л.Ф. Григорьева, Т.А. Макарова. — М.: Наука, 1966. — 184 с.

92 Currie G.P. An overview of how asbestos exposure affects the lung / G.P. Currie, S.J. Watt, N.A. Maskell // BMJ. — 2009. — Vol. 339. — b3209. DOI: 10.1136/bmj.b3209

93 Smith A.H. Chrysotile asbestos is the main cause of pleural mesothelioma / A.H. Smith, C.C. Wright // American Journal of Industrial Medicine. — 1996. — Vol. 30. — P. 252–266. DOI: 10.1002/(SICI)1097-0274(199609)30:3<252:: AID-AJIM2>3.0.CO;2-0

94 Asgharian B. Dosimetry of inhaled elongate mineral particles in the respiratory tract: The impact of shape factor / B. Asgharian, T.P. Owen, E.D. Kuempel, A.M. Jarabek // Toxicology and Applied Pharmacology. — 2018. — Vol. 361. — P. 27–35. DOI: 10.1016/j.taap.2018.05.001

95 Finkelstein M.M. Letter to the Editor re Bernstein et al: Health risk of chrysotile revisited / M.M. Finkelstein // Crit Rev Toxicol. — 2013. — Vol. 43(2). — P. 154–183.

96 Yu C.P. Intrapulmonary deposition and retention modeling of chrysotile asbestos fibers in rats / C.P. Yu, B. Asgharian, K.E. Pinkerton // Journal of Aerosol Science. — 1991. — Vol. 22. — P. 757–763. DOI: 10.1016/0021-8502(91)90068-S

97 Ramada Rodilla J.M. Fiber burden and asbestos-related diseases: an umbrella review / J.M. Ramada Rodilla, B. Calvo Cerrada, C. Serra Pujadas, G.L. Delclos, F.G. Benavides // Gaceta Sanitaria. — 2022. — Vol. 36(2). — P. 173–183. DOI: 10.1016/j.gaceta.2021.04.001

98 Dodson R.F. Asbestos content of lung tissue, lymph nodes, and pleural plaques from former shipyard workers / R.F. Dodson, M.G. Williams Jr, C.J. Corn, A. Brollo, C. Bianchi // American Review of Respiratory Diseases. — 1990. — Vol. 142. — P. 843–847. DOI: 10.1164/ajrccm/142.4.843

99 Gibbs A.R. Fibre distribution in the lungs and pleura of subjects with asbestos related diffuse pleural fibrosis / A.R. Gibbs, M. Stephens, D.M. Griffiths, B.J. Blight, F.D. Pooley // British Journal of Industrial Medicine. — 1991. — Vol. 48. — P. 762–770. DOI: 10.1136/oem.48.11.762

100 Sekido Y. Molecular pathogenesis of malignant mesothelioma / Y. Sekido // Carcinogenesis. — 2013. — Vol. 34. — P. 1413–1419. DOI: 10.1093/carcin/bgt166

101 Feder I.S. The asbestos fiber burden in human lungs: new insights into the chrysotile debate / I.S. Feder, I. Tischoff, A. Theile, I. Schmitz, R. Merget, A. Tannapfel // Eur Respir J. — 2017. — Vol. 49(6). — P. 1602534. DOI: 10.1183/13993003.02534-2016

102 Gualtieri A.F. Journey to the centre of the lung. The perspective of a mineralogist on the carcinogenic effects of mineral fibres in the lungs / A.F. Gualtieri // Journal of Hazardous Materials. — 2023. — Vol. 442. — 130077. DOI: 10.1016/j.jhazmat.2022.130077

103 Finkelstein M.M. Malignant mesothelioma among employees of a Connecticut factory that manufactured friction materials using chrysotile asbestos / M.M. Finkelstein, C. Meisenkothen // Annals of Occupational Hygiene. — 2010. — Vol. 54(6). — P. 692–696. DOI: 10.1093/annhyg/meq046

104 Frank A.L. Global use of asbestos — legitimate and illegitimate issues / A.L. Frank // J Occupational Medicine and Toxicology. — 2020. — Vol. 15. — P. 16. DOI: 10.1186/s12995-020-00267-y

105 Stayner L.T. Asbestos-related cancer and the amphibole hypothesis: 2. Stayner and colleagues respond / L.T. Stayner, D.A. Dankovic, R.A. Lemen // American Journal of Public Health. — 1997. — Vol. 87. — P. 688.

106 Stayner LT. Canada, chrysotile and cancer: Health Canada's Asbestos International Expert Panel report / LT. Stayner // Journal of Occupational and Environmental Medicine. — 2008. — Vol. 50(12). — P. 1327-1328. DOI: 10.1097/JOM.0b013e318190eff3

107 Harington J.S. The carcinogenicity of chrysotile asbestos / J.S. Harington // Annals of the New York Academy of Sciences. — 1991. — Vol. 643. — P. 465–472. DOI: 10.1111/j.1749-6632.1991.tb24496.x

108 Wagner J.C. Proceedings: Asbestos carcinogenesis / J.C. Wagner // British Journal of Cancer. — 1975. — Vol. 32. — P. 258-259. DOI: 10.1038/bjc.1975.206

109 Wagner J.C. The effects of the inhalation of asbestos in rats / J.C. Wagner, G. Berry, J.W. Skidmore, V. Timbrell // British Journal of Cancer. — 1974. — Vol. 29. — P. 252–269. DOI: 10.1038/bjc.1974.65

110 Berman D.W. The sizes, shapes, and mineralogy of asbestos structures that induce lung tumors or mesothelioma in AF/HAN rats following inhalation / D.W. Berman, K.S. Crump, E.J. Chatfield, J.M. Davis, A.D. Jones // Risk Analysis. — 1995. — Vol. 15. — P. 181–195. DOI: 10.1111/j.1539-6924.1995.tb00312.x

111 Landrigan P.J. The hazards of chrysotile asbestos: a critical review / P.J. Landrigan, W.J. Nicholson, Y. Suzuki, J. Ladou // Industrial Health. — 1999. — Vol. 37. — P. 271–280. DOI: 10.2486/indhealth.37.271

112 Davis J.M. Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats / J.M. Davis, S.T. Beckett, R.E. Bolton, P. Collings, A.P. Middleton // British Journal of Cancer. — 1978. — Vol. 37. — P. 673–688. DOI: 10.1038/bjc.1978.105

113 Hesterberg T.W. Dependence of asbestos- and mineral dust-induced transformation of mammalian cells in culture on fiber dimension / T.W. Hesterberg, J.C. Barrett // Cancer Research. — 1984. — Vol. 44. — P. 2170–2180.

114 Lenters V. A meta-analysis of asbestos and lung cancer: is better quality exposure assessment associated with steeper slopes of the exposure-response relationships? / V. Lenters, R. Vermeulen, S. Dogger, L. Stayner, L. Portengen, A. Burdorf, D. Heederik // Environmental Health Perspectives. — 2011. — Vol. 119, No. 11. — P. 1547–1555. DOI: 10.1289/ehp.1002879

115 Hodgson J.T. Mesothelioma risk from chrysotile / J.T. Hodgson, A. Darnton // Occupational and Environmental Medicine. — 2010. — Vol. 67. — P. 432. DOI: 10.1136/oem.2009.052860

116 Berman D.W. A meta-analysis of asbestos-related cancer risk that addresses fiber size and mineral type / D.W. Berman, K.S. Crump // Critical Reviews in Toxicology. — 2008. — Vol. 38, No. Suppl. 1. — P. 49–73. DOI: 10.1080/10408440802273156

117 IARC. Consensus report. Mechanisms of fiber carcinogenesis // IARC Scientific Publications. — 1996. — Vol. 140. — P. 1–9.

118 Wang J. Transformation of the released asbestos, carbon fibers and carbon nanotubes from composite materials and the changes of their potential health impacts / J. Wang, L. Schlagenhauf, A. Setyan // Journal of Nanobiotechnology. — 2017. — Vol. 156, No. 1. — P. 15. DOI: 10.1186/s12951-017-0248-7

119 Mossman B.T. Pulmonary endpoints (lung carcinomas and asbestosis) following inhalation exposure to asbestos / B.T. Mossman, M. Lippmann, T.W. Hesterberg, K.T. Kelsey, A. Barchowsky, J.C. Bonner // Journal of Toxicology and Environmental Health. Part B, Critical Reviews. — 2011. — Vol. 14(1–4). — P. 76–121. DOI: 10.1080/10937404.2011.556047

120 Gaudino G. How asbestos and other fibers cause mesothelioma / G. Gaudino, J. Xue, H. Yang // Translational Lung Cancer Research. — 2020. — Vol. 9, No. Suppl 1. — P. S39–46. DOI: 10.21037/tlcr.2020.02.01

121 Hillerdal G. Asbestos, asbestosis, pleural plaques and lung cancer / G. Hillerdal, D.W. Henderson // Scand Journal of Work and Environmental Health. — 1997. — Vol. 23(2). — P. 93–103. DOI: 10.5271/sjweh.186

122 Suzuki Y. Short, thin asbestos fibers contribute to the development of human malignant mesothelioma: pathological evidence / Y. Suzuki, S.R. Yuen, R. Ashley // International Journal of Hygiene and Environmental Health. — 2005. — Vol. 208, No. 3. — P. 201–210. DOI: 10.1016/j.ijheh.2005.01.015

123 Langer A.M. Chrysotile: its occurrence and properties as variables controlling biological effects / A.M. Langer, R.P. Nolan // Annals of Occupational Hygiene. — 1994. — Vol. 38, No. 4. — P. 427–451. DOI: 10.1093/annhyg/38.4.427

124 Wong J.Y.Y. Mesothelioma risk among those exposed to chrysotile asbestos only and mixtures that include amphibole: a case-control study in the USA, 1975–1980 / J.Y.Y. Wong, C. Rice, A. Blair, D.T. Silverman // Occupational and Environmental Medicine. — 2021. — Vol. 78, No. 3. — P. 199–202. DOI: 10.1136/oemed-2020-106665

125 Järvholm B. Asbestos and disease — a public health success story? / B. Järvholm, A. Burdorf // Scand J Work Environ Health. — 2024. — Vol. 50(2). — P. 53–60. DOI: 10.5271/sjweh.4146

126 Marsili D. Prevention of asbestos-related disease in countries currently using asbestos / D. Marsili, B. Terracini, V.S. Santana, J.P. Ramos-Bonilla, R. Pasetto, A. Mazzeo, D. Loomis, P. Comba, E. Algranti // International Journal of Environmental Research and Public Health. — 2016. — Vol. 13, No. 5. — P. 494. DOI: 10.3390/ijerph13050494

127 Kwak K. Environmental exposure to asbestos and the risk of lung cancer: a systematic review and meta-analysis / K. Kwak, D. Kang, D. Paek // Occupational and Environmental Medicine. — 2022. — Vol. 79, No. 3. — P. 207–214. DOI: 10.1136/oemed-2020-107222

128 Gwinn M.R. Meeting report: mode(s) of action of asbestos and related mineral fibers / M.R. Gwinn, D. DeVoney, A.M. Jarabek, B. Sonawane, J. Wheeler, D.N. Weissman, S. Masten, C. Thompson // Environmental Health Perspectives. — 2011. — Vol. 119. — P. 1806–1810. DOI: 10.1289/ehp.1003240

129 Bernstein D.M. Evaluation of the dose-response and fate in the lung and pleura of chrysotile-containing brake dust compared to TiO2, chrysotile, crocidolite or amosite asbestos in a 90-day quantitative inhalation toxicology study — Interim results Part 2: Histopathological examination, Confocal microscopy and collagen quantification of the lung and pleural cavity / D.M. Bernstein, B. Toth, R.A. Rogers, D.E. Kling, P. Kunzendorf, J.I. Phillips, H. Ernst // Toxicology and Applied Pharmacology. — 2020. — Vol. 387. — 114847. DOI: 10.1016/j.taap.2019.114847.

130 Toyokuni S. Genotoxicity and carcinogenicity risk of carbon nanotubes / S. Toyokuni // Advanced Drug Delivery Reviews. — 2013. — Vol. 65. — P. 2098–2110. DOI: 10.1016/j.addr.2013.05.011.

131 Van Berlo D. Carbon nanotubes: an insight into the mechanisms of their potential genotoxicity / D. Van Berlo, M.J. Clift, C. Albrecht, R.P. Schins // Swiss Medical Weekly. — 2012. — Vol. 142. — w13698. DOI: 10.4414/smw.2012.13698

132 Gupta S.S. Do carbon nanotubes and asbestos fibers exhibit common toxicity mechanisms? / S.S. Gupta, K.P. Singh, S. Gupta, M. Dusinska, Q. Rahman // Nanomaterials (Basel). — 2022. — Vol. 12, No. 10. — P. 1708. DOI: 10.3390/nano12101708.

133 Верещагин А.Л. Специфическая токсичность наночастиц (обзор) / А.Л. Верещагин, Е.А. Морозова // Южно-сибирский научный вестник. — 2022. — № 1(41). — С. 76–88.

134 Nel A. Carbon nanotube pathogenicity conforms to a unified theory for mesothelioma causation by elongate materials and fibers / A. Nel // Environ Res. — 2023. — Vol. 230. — 114580. DOI: 10.1016/j.envres.2022.114580.

135 Kane A.B. The asbestos-carbon nanotube analogy: An update / A.B. Kane, R.H. Hurt, H. Gao // Toxicology and Applied Pharmacology. — 2018. — Vol. 361. — P. 68–80. DOI: 10.1016/j.taap.2018.06.027.

136 Brims F.J. Asbestos — a legacy and a persistent problem / F.J. Brims // Journal of the Royal Naval Medical Service. — 2009. — Vol. 95(1). — P. 4–11.

137 Tossavainen A. Amphibole fibers in Chinese chrysotile asbestos / A. Tossavainen, M. Kotilainen, K. Takahashi, G. Pan, E. Vanhala // Annals of Occupational Hygiene. — 2001. — Vol. 45. — P. 145–152.

138 Шанин Н.П. Производство асбестотехнических изделий / Н.П. Шанин, М.М. Бородулин, Ю.Я. Колбовский, В.Н. Красовский. — Ленинград: Химия, 1983. — 183 с.

С.В. Яргин

Асбест және асбестке қарсы науқан: шолу

Асбестпен байланысты денсаулыққа қауіп-қатерді бағалау ауадағы асбест талшықтарының құрамы жасанды түрде арттырылған практикалық тәжірибеге негізделген. Асбест талшықтары қоршаған ортаға асбест материалдарының эрозиясы және асбест өнеркәсібіне қатысы жоқ басқа да адам әрекеттері арқылы енеді. Арнайы іздеу кезінде талшықтар денелерді сою кезінде жиі кездеседі. Көптеген зерттеулердің нәтижелері белгілі бір дәрежеде біржақты болады. Талшықтар анықталған кезде мезотелиома немесе өкпе рагы кейде асбестпен байланысты деп жіктеледі, дегенмен себеп-салдарлық байланыс дәлелденбеген күйінде қалады. Кейбір зерттеулер асбестпен кәсіби немесе тұрмыстық байланыстың тексерілмеген анемнезіне сүйенеді. Сенімді деректерді жануарлардың орта-

ша өмір сүру ұзақтығын тіркейтін эксперименттерден алуға болады. Асбесттің әртүрлі түрлерінің өзіндік сипаттамалары бар және оларды әртүрлі салаларда қолдануға болады. Мысалы, құрамында асбест бар материалдар тежегіш төсемдерін өндіруде қолданылады. Цемент пен асбест қоспасынан жасалған құрылыс бұйымдары арзан және ұзақ уақыт пайдалануға болады. Асбест бұйымдары өртке төзімді материалдар ретінде кеңінен қолданылады. Бір жағынан, асбестті пайдаланудан бас тарту қоршаған ортаға оң әсер еткенімен, бірақ екінші жағынан, оны пайдаланудан бас тарту тасымалдау мен өрттен болатын залалды арттырады, сондай-ақ құрылыс шығындарын ұлғайтады.

Кілт сөздер: асбест, хризотил, асбестке байланысты аурулар, мезотелиома, өкпе рагы, амфоболия

С.В. Яргин

Асбест и антиасбестовая кампания: обзор

Оценка рисков для здоровья, связанных с асбестом, основана на практическом опыте, когда содержание асбестовых волокон в воздухе было искусственно увеличено. Асбестовые волокна попадают в окружающую среду в результате эрозии асбестовых материалов и других видов деятельности человека, не связанных с асбестовой промышленностью. При целенаправленном поиске волокна часто обнаруживаются при вскрытии тел. Результаты многих исследований в той или иной степени предвзяты. При обнаружении волокон мезотелиома или рак легких иногда классифицируются как связанные с асбестом, хотя причинно-следственная связь остается недоказанной. Некоторые исследования опираются на непроверенный анамнез профессионального или бытового контакта с асбестом. Достоверные данные можно получить в экспериментах, регистрирующих среднюю продолжительность жизни животных. Различные виды асбеста имеют свои технические характеристики и могут использоваться в разных областях. Например, асбест используется в производстве тормозных колодок из материалов, содержащих асбест. Строительные изделия на основе смеси цемента и асбеста отличаются низкой стоимостью и длительным сроком службы. Изделия из асбеста широко используются в качестве огнеупорных материалов. С одной стороны, отказ от использования асбеста положительно сказывается на окружающей среде, но с другой стороны, отказ от его использования приведет к увеличению ущерба от транспортировки и пожаров, а также увеличит стоимость строительства.

*Ключевые слова***:** асбест; хризотил; асбест-связанные заболевания, мезотелиома, рак легкого, амфоболия

References

1 Jargin, S.V. (2023). Asbestos-related Cancer: Exaggerated Risk Perception. *Cancer Screening and Prevention, 2*(1), 51–57. DOI: 10.14218/CSP.2022.00028.

2 Hodgson, J.T., & Darnton, A. (2000). The quantitative risks of mesothelioma and lung cancer in relation to asbestos exposure. *Annals of Occupational Hygiene, 44*, 565–601.

3 Paustenbach, D., Brew, D., Ligas, S., & Heywood, J. (2021). A critical review of the 2020 EPA risk assessment for chrysotile and its many shortcomings. *Critical Reviews in Toxicology, 51*(6), 509–539. DOI: 10.1080/10408444.2021.1968337.

4 Noonan, C.W. (2017). Environmental asbestos exposure and risk of mesothelioma. *Annals of Translational Medicine, 5*(11), 234. DOI: 10.21037/atm.2017.03.74.

5 Peña-Castro, M., Montero-Acosta, M., & Saba, M. (2023). A critical review of asbestos concentrations in water and air, according to exposure sources. *Heliyon, 9*(5), e15730. DOI: 10.1016/j.heliyon.2023.e15730.

6 Ilgren, E., Van Orden, D.R., Lee, R.J., Kamiya, Y.M., & Hoskins, J.A. (2015). Further studies of Bolivian crocidolite — Part IV: Fibre width, fibre drift and their relation to mesothelioma induction: Preliminary findings. *Epidemiology Biostatistics and Public Health, 12*, e11167-1. DOI: 10.2427/11167.

7 Berry, T.A., Belluso, E., Vigliaturo, R., Gieré, R., Emmett, E.A., Testa, J.R., Steinhorn, G., & Wallis, S.L. (2022). Asbestos and other hazardous fibrous minerals: potential exposure pathways and associated health risks. *International Journal of Environmental Research and Public Health, 19*(7), 4031. DOI: 10.3390/ijerph19074031.

8 Malinconico, S., Paglietti, F., Serranti, S., Bonifazi, G., & Lonigro, I. (2022). Asbestos in soil and water: A review of analytical techniques and methods. *Journal of Hazardous Materials, 436*, 129083. DOI: 10.1016/j.jhazmat.2022.129083.

9 Casali, M., Carugno, M., Cattaneo, A., Consonni, D., Mensi, C., Cavallo, D.M., Somigliana, A., & Pesatori, A.C. (2015). Asbestos lung burden in necroscopic samples from the general population of Milan, Italy. *Annals of Occupational Hygiene, 59*, 909–921. DOI: 10.1093/annhyg/mev028.

10 Bayram, M., & Bakan, N.D. (2014). Environmental exposure to asbestos: from geology to mesothelioma. *Current Opinion in Pulmonary Medicine, 20*, 301–307. DOI: 10.1097/MCP.0000000000000053.

11 Goodman, J.E., Rhomberg, L.R., Cohen, S.M., Mundt, K.A., Case, B., Burstyn, I., Becich, M.J., & Gibbs, G. (2025). Challenges in defining thresholds for health effects: some considerations for asbestos and silica. *Front Epidemiol., 5*, 1557023. DOI: 10.3389/fepid.2025.1557023.

12 Yang, H., Testa, J.R., & Carbone, M. (2008). Mesothelioma epidemiology, carcinogenesis, and pathogenesis. *Current Treatment Options in Oncology, 9*, 147–157. DOI: 10.1007/s11864-008-0067-z

13 Kraus, T., & Jonigk, D. (2024). Mesotheliome — 30 Jahre nach dem Asbestverbot in Deutschland. *Pathologie (Heidelb), 45*(5), 305–308. DOI: 10.1007/s00292-024-01350-5.

14 Nel, A.E., Pavlisko, E.N., & Roggli, V.L. (2024). The Interplay Between the Immune System, Tumor Suppressor Genes, and Immune Senescence in Mesothelioma Development and Response to Immunotherapy. *J Thorac Oncol., 19*(4), 551–564. DOI: 10.1016/j.jtho.2023.11.017.

15 Nash, A., & Creaney, J. (2023). Genomic landscape of pleural mesothelioma and therapeutic aftermaths. *Curr Oncol Rep., 25*(12), 1515–1522. DOI: 10.1007/s11912-023-01479-1.

16 Carbone, M., Adusumilli, P.S., Alexander, H.R. Jr, Baas, P., Bardelli, F., Bononi, A., Bueno, R., Felley-Bosco, E., Galateau-Salle, F., Jablons, D., Mansfield, A.S., Minaai, M., de Perrot, M., Pesavento, P., Rusch, V., Severson, D.T., Taioli, E., Tsao, A., Woodard, G., Yang, H., Zauderer, M.G., & Pass, H.I. (2019). Mesothelioma: Scientific clues for prevention, diagnosis, and therapy. *CA: a Cancer Journal For Clinicians, 69*(5), 402–429. DOI: 10.3322/caac.21572.

17 Dipper, A., Maskell, N., & Bibby, A. (2021). Ancillary diagnostic investigations in malignant pleural mesothelioma. *Cancers (Basel), 13*(13), 3291. DOI: 10.3390/cancers13133291.

18 Donaldson, K., Poland, C.A., Murphy, F.A., MacFarlane, M., Chernova, T., & Schinwald, A. (2013). Pulmonary toxicity of carbon nanotubes and asbestos — similarities and differences. *Advanced Drug Delivery Reviews, 65*, 2078–2086. DOI: 10.1016/j.addr.2013.07.014.

19 Greim, H., Utell, M.J., Maxim, L.D., & Niebo, R. (2014). Perspectives on refractory ceramic fiber (RCF) carcinogenicity: comparisons with other fibers. *Inhalation Toxicology, 26*, 789–810. DOI: 10.3109/08958378.2014.953276.

20 Janosikova, M., Nakladalova, M., & Stepanek, L. (2023). Current causes of mesothelioma: how has the asbestos ban changed the perspective? *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub., 167*(2), 99–108. DOI: 10.5507/bp.2023.008.

21 Jasani, B., & Gibbs, A. (2012). Mesothelioma not associated with asbestos exposure. *Archives of Pathology and Laboratory Medicine, 136*, 262–267. DOI: 10.5858/arpa.2011-0039-RA.

22 Panou, V., Vyberg, M., Weinreich, U.M., Meristoudis, C., Falkmer, U.G., & Røe, O.D. (2015). The established and future biomarkers of malignant pleural mesothelioma. *Cancer Treatment Reviews, 41*, 486–495. DOI: 10.1016/j.ctrv.2015.05.001.

23 Røe, O.D. & Stella, G.M. (2015). Malignant pleural mesothelioma: history, controversy and future of a manmade epidemic. *European Respiratory Review, 24*, 115–131. DOI: 10.1183/09059180.00007014.

24 Røe, O.D., Anderssen, E., Helge, E., Pettersen, C.H., Olsen, K.S., Sandeck, H., Haaverstad, R., Lundgren, S., & Larsson, E. (2009). Genome-wide profile of pleural mesothelioma versus parietal and visceral pleura: the emerging gene portrait of the mesothelioma phenotype. *PLoS One*, e6554. DOI: 10.1371/journal.pone.0006554.

25 Rossini, M., Rizzo, P., Bononi, I., Clementz, A., Ferrari, R., Martini, F., & Tognon, M.G. (2018). New perspectives on diagnosis and therapy of malignant pleural mesothelioma. *Frontiers of Oncology, 8*, 91. DOI: 10.3389/fonc.2018.00091.

26 Carbone, M., Gazdar, A., & Butel, J.S. (2020). SV40 and human mesothelioma. *Translational Lung Cancer Research, 9*(Suppl 1), S47–S59. DOI: 10.21037/tlcr.2020.02.03.

27 Testa, J.R., Carbone, M., Hirvonen, A., Khalili, K., Krynska, B., Linnainmaa, K., Pooley, F.D., Rizzo, P., Rusch, V., & Xiao, G.H. (1998). A multi-institutional study confirms the presence and expression of simian virus 40 in human malignant mesotheliomas. *Cancer Research, 58*(20), 4505–4509.

28 Cicala, C., Pompetti, F., & Carbone, M. (1993). SV40 induces mesotheliomas in hamsters. *American Journal of Pathology, 142*, 1524–1533.

29 Mazzoni, E., Bononi, I., Rotondo, J.C., Mazziotta, C., Libener, R., Guaschino, R., Gafà, R., Lanza, G., Martini, F., & Tognon, M. (2022). Sera from patients with malignant pleural mesothelioma tested positive for IgG antibodies against SV40 large t antigen: the viral oncoprotein. *J. Oncol., 2022*, 7249912. DOI: 10.1155/2022/7249912.

30 Elovskaya, L.T. (1997). Antiasbestovaia kampaniia i konferentsiia po probleme «Asbest i zdorove» [Anti-asbestos campaign and conference on "Asbestos and health"]. *Meditsina Truda i Promyshlennaia Ekologiia — Occupational medicine and industrial ecology*, 9, 16–21 [in Russian].

31 Kazantsev, V.A. (2004). Primenenie bronkhologicheskoi sanatsii dlia lecheniia bolnykh vnebolnichnymi pnevmoniiami [The use of bronchological sanation for treatment of community-acquired pneumonia]. *14-i Natsionalnyi Kongress — 14th National Congress*, 361. Moscow: Russian Respiratory Society [in Russian].

32 Likhacheva, E.I., Iarina, A.L., Vagina, E.R., Klimina, M.S., Obukhova, T.Yu., Dovgoliuk, T.A., & Kashanskii, S.V. (2000). Klinicheskie osobennosti zabolevanii legkikh ot vozdeistviia pyli khrizotil-asbesta [Clinical features of pulmonary diseases caused by chrysotile asbestos dust]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational health and industrial ecology*, 11, 30–33 [in Russian].

33 Milishnikova, V.V., Loshchilov, I.U., Gladkova, E.V., Aksenova, A.O., & Turkina, L.A. (1990). Endoskopicheskaia i morfologicheskaia kharakteristika bronkhov i legkikh pri asbestoze i pylevom bronkhite u rabotnikov asbotekstilnoi promyshlennosti [Endoscopic and morphological characteristics of the bronchi and lungs in asbestosis and dust-induced bronchitis in asbestos-textile industry workers]. *Gigiena truda i professionalnye zabolevaniia — Work hygiene and occupational diseases*, 7, 19–22 [in Russian].

34 Lorenzini, E., Ciarrocchi, A., & Torricelli, F. (2021). Molecular fingerprints of malignant pleural mesothelioma: not just a matter of genetic alterations. *Journal of Clinical Medicine, 10*(11), 2470. DOI: 10.3390/jcm10112470.

35 Carbone, M., & Yang, H. (2017). Mesothelioma: recent highlights. *Annals of Translational Medicine, 5*(11), 238. DOI: 10.21037/atm.2017.04.29.

36 Chen, Z., Gaudino, G., Pass, H.I., Carbone, M., & Yang, H. (2017). Diagnostic and prognostic biomarkers for malignant mesothelioma: an update. *Translational Lung Cancer Research, 6*, 259–269. DOI: 10.21037/tlcr.2017.05.06.

37 Goldberg, M., Imbernon, E., Rolland, P., Gilg Soit, Ilg, A., Savès, M., de Quillacq, A., Frenay, C., Chamming's, S., Arveux, P., Boutin, C., Launoy, G., Pairon, J.C., Astoul, P., Galateau-Sallé, F., & Brochard, P. (2006). The French national mesothelioma surveillance program. *Occupational and Environmental Medicine, 63*, 390–395. DOI: 10.1136/oem.2005.023200.

38 Lucà, S., Pignata, G., Cioce, A., Salzillo, C., De Cecio, R., Ferrara, G., Della Corte, C.M., Morgillo, F., Fiorelli, A., Montella, M., & Franco, R. (2025). Diagnostic challenges in the pathological approach to pleural mesothelioma. *Cancers (Basel), 17*(3), 481. DOI: 10.3390/cancers17030481.

39 Creaney, J., Dick, I.M., & Robinson, B.W. (2015). Discovery of new biomarkers for malignant mesothelioma. *Curr Pulmonology Reports, 4*, 15–21. DOI: 10.1007/s13665-015-0106-8.

40 Ho, M., Bera, T.K., Willingham, M.C., Onda, M., Hassan, R., FitzGerald, D., & Pastan, I. (2007). Mesothelin expression in human lung cancer. *Clinical Cancer Research, 13*(5), 1571–1575. DOI: 10.1158/1078-0432.CCR-06-2161.

41 Bibby, A.C., Tsim, S., Kanellakis, N., Ball, H., Talbot, D.C., Blyth, K.G., Maskell, N.A., & Psallidas, I. (2016). Malignant pleural mesothelioma: an update on investigation, diagnosis and treatment. *European Respiratory Review, 25*, 472–486. DOI: 10.1183/16000617.0063-2016.

42 Grigoriu, B.D., Grigoriu, C., Chahine, B., Gey, T., & Scherpereel, A. (2009). Clinical utility of diagnostic markers for malignant pleural mesothelioma. *Monaldi Archive of Chest Diseases, 71*(1), 31–38. DOI: 10.4081/monaldi.2009.374.

43 Pantazopoulos, I., Boura, P., Xanthos, T., & Syrigos, K. (2013). Effectiveness of mesothelin family proteins and osteopontin for malignant mesothelioma. *European Respiratory Journal, 41*(3), 706–715. DOI: 10.1183/09031936.00226111.

44 Harris, E.J.A., Musk, A., de Klerk, N., Reid, A., Franklin, P., & Brims, F.J.H. (2019). Diagnosis of asbestos-related lung diseases. *Expert Review of Respiratory Medicine, 13*(3), 241–249. DOI: 10.1080/17476348.2019.1568875.

45 Lindholm, P.M., Salmenkivi, K., Vauhkonen, H., Nicholson, A.G., Anttila, S., Kinnula, V.L., & Knuutila, S. (2007). Gene copy number analysis in malignant pleural mesothelioma using oligonucleotide array CGH. *Cytogenetic and Genome Research, 119*, 46–52. DOI: 10.1159/000109618.

46 Musti, M., Kettunen, E., Dragonieri, S., Lindholm, P., Cavone, D., Serio, G., & Knuutila, S. (2006). Cytogenetic and molecular genetic changes in malignant mesothelioma. *Cancer Genetics and Cytogenetics, 170*, 9–15. DOI: 10.1016/j.cancergencyto.2006.04.011.

47 Cersosimo, F., Barbarino, M., Lonardi, S., Vermi, W., Giordano, A., Bellan, C., & Giurisato, E. (2021). Mesothelioma malignancy and the microenvironment: molecular mechanisms. *Cancers (Basel), 13*(22), 5664. DOI: 10.3390/cancers13225664.

48 Vandenhoeck, J., van Meerbeeck, J.P., Fransen, E., Raskin, J., Van Camp, G., Op de Beeck, K., & Lamote, K. (2021). DNA methylation as a diagnostic biomarker for malignant mesothelioma: a systematic review and meta-analysis. *Journal of Thoracic Oncology, 16*(9), 1461–1478. DOI: 10.1016/j.jtho.2021.05.015.

49 Zahiu, T., Mihu, C.M., Bosca, B.A., Mărginean, M., Mocan, L.P., Ștefan, R.A., Suflețel, R.T., Mihu, C., & Melincovici, C.S. (2025). Molecular Insights into Pleural Mesothelioma: Unveiling Pathogenic Mechanisms and Therapeutic Opportunities. *Diagnostics (Basel), 15*(11), 1323. DOI: 10.3390/diagnostics15111323.

50 Ferrari, L., Carugno, M., Mensi, C., & Pesatori, A.C. (2020). Circulating epigenetic biomarkers in malignant pleural mesothelioma: state of the art and critical evaluation. *Frontiers of Oncology, 10*, 445. DOI: 10.3389/fonc.2020.00445.

51 Wolff, H., Vehmas, T., Oksa, P., Rantanen, J., & Vainio, H. (2015). Asbestos, asbestosis, and cancer, the Helsinki criteria for diagnosis and attribution 2014: recommendations. *Scandinavian Journal of Work and Environmental Health, 41*(1), 5–15. DOI: 10.5271/sjweh.3462.

52 Rossi, G., Davoli, F., Poletti, V., Cavazza, A., & Lococo, F. (2021). When the diagnosis of mesothelioma challenges textbooks and guidelines. *Journal of Clinical Medicine, 10*(11), 2434. DOI: 10.3390/jcm10112434.

53 Schillebeeckx, E., van Meerbeeck, J.P., & Lamote, K. (2021). Clinical utility of diagnostic biomarkers in malignant pleural mesothelioma: a systematic review and meta-analysis. *European Respiratory Review, 30*(162), 210057. DOI: 10.1183/16000617.0057-2021.

54 Izmerov, N.F., & Kovalevsky, E.V. (2004). Normativnoe obespechenie kontroliruemogo ispolzovaniia asbestosoderzhashchikh materialov v stroitelstve [Regulations of controlled use of asbestos-containing materials in construction industry]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational health and industrial ecology*, 5, 5–12 [in Russian].

55 Neiman, S.M., Vezentsev, A.I., & Kashanskii, S.V. (2006). *O bezopasnosti asbestotsementnykh materialov i izdelii* [On the safety of asbestos-cement materials and products]. Moscow: Stroimaterialy [in Russian].

56 Kogan, F.M., Kashanskii, S.V., Plotko, E.G., Berzin, S.A., & Bogdanov, G.B. (1993). Vliianie nizkoi kontsentratsii asbestosoderzhashchei pyli [Effect of low concentration of asbestos-containing dust]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational medicine and industrial ecology*, 5-6, 6–10 [in Russian].

57 Shtol, A.V., Plotko, E.G., & Seliankina, K.P. (2000). Zagriaznenie atmosfernogo vozdukha asbestosoderzhashchei pyliu i zdorove detskogo naseleniia [Air pollution with asbestos-containing dust and the health of the child population]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational health and industrial ecology*, 11, 10–13.

58 Tsurikova, G.V., Spitsyn, V.A., Gladkova, E.V., & Minaeva, O.P. (1992). Biodemograficheskie parametry kak indikatory geneticheskoi adaptatsii k vrednym professionalnym faktoram na primere asbesta [Biodemographic parameters as indicators of genetic adaptation to harmful occupational factors using asbestos as an example]. *Gigiena truda i professionalnye zabolevaniia — Work hygiene and occupational diseases,* 6, 28–30 [in Russian].

59 Kashansky, S.V., Domnin, S.G., Plotko, E.G., Kuzmin, S.V., Selyankina, S.V., & Likhacheva, E.I. (2004). Sovremennye problemy asbesta i perspektivnye napravleniia issledovanii [Current problems of asbestos and promising areas of research]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational health and industrial ecology,* 9, 16–19 [in Russian].

60 Krasovskii, G.N., & Egorova, N.A. (1985). Asbest i kachestvo pitevoi vody [Asbestos and the quality of drinking water]. *Gigiena i sanitariia — Hygiene and Sanitation,* 3, 64–67 [in Russian].

61 Krasovskii, G.N., & Mozhaev, E.A. (1993). Asbest v pitevoi vode (obzor) [Asbestos in drinking water (review)]. *Gigiena i Sanitariia — Hygiene and sanitation,* 6, 20–22 [in Russian].

62 Repina, Zh.V., Chemyakina, N.A., & Tarskaya-Lapteva, E.G. (2009). *Khrizotiltsementnye stroitelnie materialy. Oblasti ispolzovaniia* [Chrysotile cement building materials. Areas of use]. Yekaterinburg: AMB [in Russian].

63 Kogan, F.M. (2002). Osnovnye rezultaty issledovanii po probleme «Asbest-Zdorove» [Main results of the study on the problem of "Asbestos-Health"]. *Profilaktika asbestoobuslovlennykh zabolevanii. Sbornik publikatsii: Asbest: Asbestovaia assotsiatsiia — In Prevention of asbestos-related diseases: Collection of publications. Asbestos: Asbestos association* (pp. 7–9) [in Russian].

64 Kaptsov, V.A., Kashanskii, S.V., Domnin, S.G., Tikhova, T.S., Trofimova, E.V., Novoselova, T.A., & Bogdanov, G.B. (2003). Zheleznodorozhnoe ispolzovanie asbestosoderzhashchego shchebnia: ekologo-gigienicheskie aspekty [Railway use of asbestos-containing rubble: environmental hygienic aspects]. *Gigiena i Sanitariia — Hygiene and sanitation,* 5, 11–15.

65 Kashanskii, S.V., & Kogan, F.M. (1995). Opasnost razvitiia raka legkikh pri proizvodstve asbestovykh panelei [The danger of developing lung cancer in the manufacture of asbestos panels]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational health and industrial ecology,* 5, 19–22 [in Russian].

66 Pylev, L.N., Smirnova, O.V., & Vezentsev, A.I. (2011). Khrizotiltsementnoe proizvodstvo — istochnik kantserogennoi opasnosti dlia cheloveka? [Chrysotile cement production — a source of carcinogenic hazard to humans?]. *Toksikologicheskii vestnik — Toxicological Review,* 4, 46–50 [in Russian].

67 Yatsenko, A.S., Kogan, F.M., Fomina, A.S., Zykov, V.A., Nikitina, O.V., Vanchugova, N.N., Bogdanov, G.B., Aliamovsky, S.I., & Perelyaeva, L.A. (1994). O vzaimosviazi mezhdu biologicheskoi agressivnostiu i nekotorymi fiziko-khimicheskimi svoistvami promyshlennoi pyli, voznikaiushchei pri proizvodstve i primenenii friktsionnykh izdelii [On the relationship between biological aggressiveness and certain physicochemical properties of industrial dust generated during the production and use of friction products]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational health and industrial ecology,* 12, 29–33 [in Russian].

68 Kovalevskii, E.V. (2009). Gigienicheskaia otsenka primeneniia asbestosoderzhashchikh friktsionnykh izdelii [Hygienic evaluation of asbestos-containing friction goods application]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational health and industrial ecology,* 7, 1–6 [in Russian].

69 Yatsenko, A.S., & Kogan, F.M. (1990). Professionalnaia zabolevaemost i smertnost ot zlokachestvennykh novoobrazovanii sredi lits, imeiushchikh professionalnyi kontakt s asbestovoi pyliu [Occupational morbidity and mortality from malignant neoplasms among persons professionally exposed to asbestos dust]. *Gigiena truda i professionalnye zabolevaniia — Occupational Hygiene and Occupational Diseases,* 2, 10–12 [in Russian].

70 Yatsenko, A.S., Kogan, F.M., Elnichnykh, L.N., & Remizova, I.I. (1991). Sravnitelnoe issledovanie fibrinogennoi aktivnosti pyli pri proizvodstve asbestoformovannykh detalei [Comparative study of fibrinogen activity of dust in the production of asbestos-molded parts]. *Gigiena i sanitariia — Hygiene and sanitation,* 8, 27–29 [in Russian].

71 Izmerov, N.F., Elovskaya, L.T., Milishnikova, V.V., Burmistrova, T.B., & Kovalevsky, E.V. (1998). Khrizotil-asbest v Rossii: nekotorye rezultaty i perspektivnye napravleniia issledovanii [Chrysotile asbestos in Russia: certain results and promising research directions]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational health and industrial ecology,* 10, 1–7 [in Russian].

72 Kashanskii, S.V. (2008). Mezotelioma v Rossii: sistemnyi obzor 3576 opublikovannykh sluchaev s pozitsii meditsiny truda [Mesothelioma in Russia: systematic review of 3576 published cases from occupational medicine viewpoint]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational health and industrial ecology,* 3, 15–21 [in Russian].

73 Kashanskii, S.V., Zhetpisbaev, B.A., Il'derbaev, O.Z., & Ermenbai, O.T. (2008). Mezotelioma v Respublike Kazakhstan: obzor [Mesothelioma in the Republic of Kazakhstan: a review]. *Gigiena i sanitariia — Hygiene and sanitation,* 5, 13–17 [in Russian].

74 Kogan, F.M. (1995). *Sovremennye predstavleniia o bezopasnosti asbesta* [Modern concept of asbestos safety]. Yekaterinburg: Argo [in Russian].

75 Pylev, L.N., Kogan, F.M., & Kulagina, T.F. (1988). Kantserogennaia aktivnost asbestotsementnoi pyli [Carcinogenic activity of asbestos-cement dust]. *Gigiena truda i professionalnye zabolevaniia — Work hygiene and occupational diseases*, 7, 55–57 [in Russian].

76 Pylev, L.N., Smirnova, O.V., Vasilyeva, L.A., Khrustalev, S.A., Vezencev, A.I., Gudkova, E.A., & Naumova, L.N. (2010). Eksperimentalnoe obosnovanie kantserogennoi opasnosti asbestotsementnoi promyshlennosti i ee produktsii [Experimental evidence of the carcinogenic hazard of the asbestos cement industry and its products]. *Gigiena i sanitariia — Hygiene and sanitation,* 6, 61–65 [in Russian].

77 Troitskaya, N.A. (1993). Sravnitelnoe issledovanie tsitotoksichnosti pyli uglerodnykh volokon i drugikh voloknistykh materialov [Comparative study of cytotoxicity of carbon fiber dust and other fibrous materials]. *Gigiena i sanitariia — Hygiene and sanitation*, 3, 28–30 [in Russian].

78 Kogan, F.M. (2002). K voprosu o normirovanii asbestsoderzhashchikh pylei v vozdukhe rabochikh pomeshchenii [On the issue of standardization of asbestos-containing dusts in the air of working premises]. *Profilaktika asbestoobuslovlennykh zabolevanii: Sbornik publikatsii: Asbestos: Asbestovaia assotsiatsiia —* In *Prevention of asbestos-related diseases: Collection of publications: Asbestos: Asbestos association* (pp. 57–63) [in Russian].

79 Kashansky, S.V., Kogan, F.M., Malysheva, L.G., & Zykova, V.A. (1994). Sravnitelnaia otsenka fibrogeneza i toksichnosti asbestosoderzhashchikh teploizoliatsionnykh materialov [Comparative evaluation of fibrogenesis and toxicity of asbestos-containing heat-insulating materials]. *Meditsina truda i promyshlennaia ekologiia — Russian journal of occupational health and industrial ecology*, 1, 17–21 [in Russian].

80 Pylev, LN. (1987). Rol modifitsiruiushchikh faktorov v kantserogennom deistvii asbesta i asbestosoderzhashchikh pylei [The role of modifying factors in the carcinogenic effect of asbestos and asbestos-containing dusts]. *Eksperimentalnaia onkologiia — Experimental Oncology*, 9(5), 14–17 [in Russian].

81 Bernstein, D.M. (2014). The health risk of chrysotile asbestos. *Current Opinion in Pulmonary Medicine, 20*, 366–370. DOI: 10.1097/MCP.0000000000000064.

82 Coin, P.G., Roggli, V.L., & Brody, A.R. (1994). Persistence of long, thin chrysotile asbestos fibers in the lungs of rats. *Environ Health Perspective, 102*, 197–199. DOI: 10.1289/ehp.94102s5197.

83 Kohyama, N., & Suzuki, Y. (1991). Analysis of asbestos fibers in lung parenchyma, pleural plaques, and mesothelioma tissues of North American insulation workers. *Annals of the New York Academy of Sciences, 643*, 27–52. DOI: 10.1111/j.1749-6632.1991.tb24442.x.

84 Nicholson, W.J. (1991). Comparative dose-response relationships of asbestos fiber types: magnitudes and uncertainties. *Annals of the New York Academy of Sciences, 643*, 74–84. DOI: 10.1111/j.1749-6632.1991.tb24446.x.

85 Sebastien, P., Janson, X., Gaudichet, A., Hirsch, A., & Bignon, J. (1980). Asbestos retention in human respiratory tissues: comparative measurements in lung parenchyma and in parietal pleura. *IARC Scientific Publications, 30*, 237–246.

86 Stayner, L.T., Dankovic, D.A., & Lemen, R.A. (1996). Occupational exposure to chrysotile asbestos and cancer risk: a review of the amphibole hypothesis. *American Journal of Public Health, 86*, 179–186. DOI: 10.2105/ajph.86.2.179.

87 Suzuki, Y., & Yuen, S.R. (2002). Asbestos fibers contributing to the induction of human malignant mesothelioma. *Annals of the New York Academy of Sciences, 982*, 160–176. DOI: 10.1111/j.1749-6632.2002.tb04931.x.24.

88 Pezerat, H. (2009). Chrysotile biopersistence: the misuse of biased studies. *International Journal of Occupational and Environmental Health, 15*, 102–106. DOI: 10.1179/107735209799449770.

89 Koigeldinova, Sh.S., Ibraev, S.A., Zhuzbaeva, G.O., & Kasymova, A.K. (2015). Sovremennyi vzgliad na problemu professionalnykh zabolevanii legkikh ot vozdeistviia khrizotil-asbesta [A modern view on the problem of occupational lung diseases caused by exposure to chrysotile asbestos]. *Vestnik Karagandinskogo universiteta. Seriia «Biologiia. Meditsina. Geografiia» — Bulletin of Karaganda University. Series Biology. Medicine. Geography*, *79*(3), 122–131 [in Russian].

90 Larsen, G. (1989). Experimental data on in vitro fiber solubility. *IARC Scientific Publications, 90*, 134–139.

91 Fedoseev, A.D., Grigorieva, L.F., & Makarova, T.A. (1966). *Voloknistye silikaty. Prirodnye i sinteticheskie asbesty* [Fibrous silicates. Natural and synthetic asbes]. Moscow: Nauka [in Russian].

92 Currie, G.P., Watt, S.J., & Maskell, N.A. (2009). An overview of how asbestos exposure affects the lung. *BMJ, 339*, b3209. DOI: 10.1136/bmj.b3209.

93 Smith, A.H., & Wright, C.C. (1996). Chrysotile asbestos is the main cause of pleural mesothelioma. *American Journal of Industrial Medicine, 30*, 252–266. DOI: 10.1002/(SICI)1097-0274(199609)30:3<252:: AID-AJIM2>3.0.CO;2-0.

94 Asgharian, B., Owen, T.P., Kuempel, E.D., & Jarabek, A.M. (2018). Dosimetry of inhaled elongate mineral particles in the respiratory tract: The impact of shape factor. *Toxicology and Applied Pharmacology, 361*, 27–35. DOI: 10.1016/j.taap.2018.05.001.

95 Finkelstein, M.M. (2013). Letter to the Editor re Bernstein et al: Health risk of chrysotile revisited. *Crit Rev Toxicol, 43*(2), 154–183.

96 Yu, C.P., Asgharian, B., & Pinkerton, K.E. (1991). Intrapulmonary deposition and retention modeling of chrysotile asbestos fibers in rats. *Journal of Aerosol Science, 22*, 757–763. DOI: 10.1016/0021-8502(91)90068-S.

97 Ramada Rodilla, J.M., Calvo Cerrada, B., Serra Pujadas, C., Delclos, G.L., & Benavides, F.G. (2022). Fiber burden and asbestos-related diseases: an umbrella review. *Gaceta Sanitaria, 36*(2), 173–183. DOI: 10.1016/j.gaceta.2021.04.001.

98 Dodson, R.F., Williams, M.G. Jr, Corn, C.J., Brollo, A., & Bianchi, C. (1990). Asbestos content of lung tissue, lymph nodes, and pleural plaques from former shipyard workers. *American Review of Respiratory Diseases, 142*, 843–847. DOI: 10.1164/ajrccm/142.4.843.

99 Gibbs, A.R., Stephens, M., Griffiths, D.M., Blight, B.J., & Pooley, F.D. (1991). Fibre distribution in the lungs and pleura of subjects with asbestos related diffuse pleural fibrosis. *British Journal of Industrial Medicine, 48*, 762–770. DOI: 10.1136/oem.48.11.762.

100 Sekido, Y. (2013). Molecular pathogenesis of malignant mesothelioma. *Carcinogenesis, 34*, 1413–1419. DOI: 10.1093/carcin/bgt166.

101 Feder, I.S., Tischoff, I., Theile, A., Schmitz, I., Merget, R., & Tannapfel, A. (2017). The asbestos fiber burden in human lungs: new insights into the chrysotile debate. *Eur Respir J., 49*(6), 1602534. DOI: 10.1183/13993003.02534-2016.

102 Gualtieri, A.F. (2023). Journey to the centre of the lung. The perspective of a mineralogist on the carcinogenic effects of mineral fibres in the lungs. *Journal of Hazardous Materials, 442*, 130077. DOI: 10.1016/j.jhazmat.2022.130077.

103 Finkelstein, M.M., & Meisenkothen, C. (2010). Malignant mesothelioma among employees of a Connecticut factory that manufactured friction materials using chrysotile asbestos. *Annals of Occupational Hygiene, 54*(6), 692–696. DOI: 10.1093/annhyg/meq046.

104 Frank, A.L. (2020). Global use of asbestos — legitimate and illegitimate issues. *J Occupational Medicine and Toxicology, 15*, 16. DOI: 10.1186/s12995-020-00267-y.

105 Stayner, L.T., Dankovic, D.A., & Lemen, R.A. (1997). Asbestos-related cancer and the amphibole hypothesis: 2. Stayner and colleagues respond. *American Journal of Public Health, 87*, 688.

106 Stayner, L.T. (2008). Canada, chrysotile and cancer: Health Canada's Asbestos International Expert Panel report. *Journal of Occupational and Environmental Medicine, 50*(12), 1327-1328. DOI: 10.1097/JOM.0b013e318190eff3.

107 Harington, J.S. (1991). The carcinogenicity of chrysotile asbestos. *Annals of the New York Academy of Sciences, 643*, 465–472. DOI: 10.1111/j.1749-6632.1991.tb24496.x.

108 Wagner, J.C. (1975). Proceedings: Asbestos carcinogenesis. *British Journal of Cancer, 32*, 258-259. DOI: 10.1038/bjc.1975.206.

109 Wagner, J.C., Berry, G., Skidmore, J.W., & Timbrell, V. (1974). The effects of the inhalation of asbestos in rats. *British Journal of Cancer, 29*, 252–269. DOI: 10.1038/bjc.1974.65.

110 Berman, D.W., Crump, K.S., Chatfield, E.J., Davis, J.M., & Jones, A.D. (1995). The sizes, shapes, and mineralogy of asbestos structures that induce lung tumors or mesothelioma in AF/HAN rats following inhalation. *Risk Analysis, 15*, 181–195. DOI: 10.1111/j.1539-6924.1995.tb00312.x.

111 Landrigan, P.J., Nicholson, W.J., Suzuki, Y., & Ladou, J. (1999). The hazards of chrysotile asbestos: a critical review. *Industrial Health, 37*, 271–280. DOI: 10.2486/indhealth.37.271.

112 Davis, J.M., Beckett, S.T., Bolton, R.E., Collings, P., & Middleton. A.P. (1978). Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats. *British Journal of Cancer, 37*, 673–688. DOI: 10.1038/bjc.1978.105.

113 Hesterberg, T.W., & Barrett, J.C. (1984). Dependence of asbestos- and mineral dust-induced transformation of mammalian cells in culture on fiber dimension. *Cancer Research, 44*, 2170–2180.

114 Lenters, V., Vermeulen, R., Dogger, S., Stayner, L., Portengen, L., Burdorf, A., & Heederik, D. (2011). A meta-analysis of asbestos and lung cancer: is better quality exposure assessment associated with steeper slopes of the exposure-response relationships? *Environmental Health Perspectives, 119*(11), 1547–1555. DOI: 10.1289/ehp.1002879.

115 Hodgson, J.T., & Darnton, A. (2010). Mesothelioma risk from chrysotile. *Occupational and Environmental Medicine, 67*, 432. DOI: 10.1136/oem.2009.052860.

116 Berman, D.W., & Crump, K.S. (2008). A meta-analysis of asbestos-related cancer risk that addresses fiber size and mineral type. *Critical Reviews in Toxicology, 38*(1), 49–73. DOI: 10.1080/10408440802273156.

117 (1996). IARC. Consensus report. Mechanisms of fiber carcinogenesis. *IARC Scientific Publications, 140*, 1–9.

118 Wang, J., Schlagenhauf, L., & Setyan, A. (2017). Transformation of the released asbestos, carbon fibers and carbon nanotubes from composite materials and the changes of their potential health impacts. *Journal of Nanobiotechnology, 15*(1), 15. DOI: 10.1186/s12951-017-0248-7.

119 Mossman, B.T., Lippmann, M., Hesterberg, T.W., Kelsey, K.T., Barchowsky, A., & Bonner, J.C. (2011). Pulmonary endpoints (lung carcinomas and asbestosis) following inhalation exposure to asbestos. *Journal of Toxicology and Environmental Health. Part B, Critical Reviews, 14*(1–4), 76–121. DOI: 10.1080/10937404.2011.556047.

120 Gaudino, G., Xue, J., & Yang, H. (2020). How asbestos and other fibers cause mesothelioma. *Translational Lung Cancer Research, 9*(Suppl 1), S39–46. DOI: 10.21037/tlcr.2020.02.01.

121 Hillerdal, G., & Henderson, D.W. (1997). Asbestos, asbestosis, pleural plaques and lung cancer. *Scand Journal of Work and Environmental Health, 23*(2), 93–103. DOI: 10.5271/sjweh.186.

122 Suzuki, Y., Yuen, S.R., & Ashley, R. (2005). Short, thin asbestos fibers contribute to the development of human malignant mesothelioma: pathological evidence. International *Journal of Hygiene and Environmental Health, 208*(3), 201–210. DOI: 10.1016/j.ijheh.2005.01.015.

123 Langer, A.M., & Nolan, R.P. (1994). Chrysotile: its occurrence and properties as variables controlling biological effects. *Annals of Occupational Hygiene, 38*(4), 427–451. DOI: 10.1093/annhyg/38.4.427.

124 Wong, J.Y.Y., Rice, C., Blair, A., & Silverman, D.T. (2021). Mesothelioma risk among those exposed to chrysotile asbestos only and mixtures that include amphibole: a case-control study in the USA, 1975–1980. *Occupational and Environmental Medicine, 78*(3), 199–202. DOI: 10.1136/oemed-2020-106665.

125 Järvholm, B., & Burdorf, A. (2024). Asbestos and disease — a public health success story? *Scand J Work Environ Health, 50*(2), 53–60. DOI: 10.5271/sjweh.4146.

126 Marsili, D., Terracini, B., Santana, V.S., Ramos-Bonilla, J.P., Pasetto, R., Mazzeo, A., Loomis, D., Comba, P., & Algranti, E. (2016). Prevention of asbestos-related disease in countries currently using asbestos. *International Journal of Environmental Research and Public Health, 13*(5), 494. DOI: 10.3390/ijerph13050494.

127 Kwak, K., Kang, D., & Paek, D. (2022). Environmental exposure to asbestos and the risk of lung cancer: a systematic review and meta-analysis. *Occupational and Environmental Medicine, 79*(3), 207–214. DOI: 10.1136/oemed-2020-107222.

128 Gwinn, M.R., DeVoney, D., Jarabek, A.M., Sonawane, B., Wheeler, J., Weissman, D.N., Masten, S., & Thompson, C. (2011). Meeting report: mode(s) of action of asbestos and related mineral fibers. *Environmental Health Perspectives, 119*, 1806–1810. DOI: 10.1289/ehp.1003240.

129 Bernstein, D.M., Toth, B., Rogers, R.A., Kling, D.E., Kunzendorf, P., Phillips, J.I., & Ernst, H. (2020). Evaluation of the dose-response and fate in the lung and pleura of chrysotile-containing brake dust compared to TiO2, chrysotile, crocidolite or amosite asbestos in a 90-day quantitative inhalation toxicology study — Interim results Part 2: Histopathological examination, Confocal microscopy and collagen quantification of the lung and pleural cavity. *Toxicology and Applied Pharmacology, 387*, 114847. DOI: 10.1016/j.taap.2019.114847.

130 Toyokuni, S. (2013). Genotoxicity and carcinogenicity risk of carbon nanotubes. *Advanced Drug Delivery Reviews, 65*, 2098–2110. DOI: 10.1016/j.addr.2013.05.011.

131 Van Berlo, D., Clift, M.J., Albrecht, C., & Schins, R.P. (2012). Carbon nanotubes: an insight into the mechanisms of their potential genotoxicity. *Swiss Medical Weekly, 142*, w13698. DOI: 10.4414/smw.2012.13698.

132 Gupta, S.S., Singh, K.P., Gupta, S., Dusinska, M., & Rahman, Q. (2022). Do carbon nanotubes and asbestos fibers exhibit common toxicity mechanisms? *Nanomaterials (Basel), 12*(10), 1708. DOI: 10.3390/nano12101708.

133 Vereshchagin, A.L., & Morozova E.A. (2022). Spetsificheskaia toksichnost nanochastits (obzor) [Specific toxicity of nanoparticles (review)]. *Yuzhno-sibirskii nauchnyi vestnik — South Siberian Scientific Bulletin,* 1(41), 76–88 [in Russian].

134 Nel, A. (2023). Carbon nanotube pathogenicity conforms to a unified theory for mesothelioma causation by elongate materials and fibers. *Environ Res., 230*, 114580. DOI: 10.1016/j.envres.2022.114580.

135 Kane, A.B., Hurt, R.H., & Gao, H. (2018). The asbestos-carbon nanotube analogy: An update. *Toxicology and Applied Pharmacology, 361*, 68–80. DOI: 10.1016/j.taap.2018.06.027.

136 Brims, F.J. (2009). Asbestos — a legacy and a persistent problem. *Journal of the Royal Naval Medical Service, 95*(1), 4–11.

137 Tossavainen, A., Kotilainen, M., Takahashi, K., Pan, G., & Vanhala, E. (2001). Amphibole fibers in Chinese chrysotile asbestos. *Annals of Occupational Hygiene, 45*, 145–152.

138 Shanin, N.P., Borodulin, M.M., Kolbovsky, Yu.Ya., & Krasovsky, V.N. (1983). *Proizvodstvo asbestotekhnicheskikh izdelii* [Production of asbestos technical products]. Leningrad: Khimia [in Russian].

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Diversity and characteristics of the genus *Artemisia* L. growing in Jetisu region

The article presents information on the species composition and distribution of the genus *Artemisia* L. in the Jetisu region based on an analysis of literature sources and herbarium materials. A total of 81 Artemisia species are recorded across Kazakhstan, occurring in deserts, steppe zones, and mountainous regions, with more than 35 species identified in the Jetisu region. An extensive review of the species found in the Jetisu region and the Jetisu Alatau was conducted, and the herbarium data of the wormwood species found in the study area (AA, AFKNU, MW funds) were accurately presented. In addition, using herbarium samples and literary data, the GPS coordinates of the wormwood found in the Jetisu were determined. The conducted study allowed to clarify and supplement information on the distribution area of the genus *Artemisia* L.

Keywords: wormwood, *Artemisia* L., endemic, distribution, herbarium, Kazakhstan, Jetisu region, Zhetysu Alatau.

Introduction

Wormwood (*Artemisia* L.) more than 500 species are distributed worldwide in the temperate climate zone of Eurasia and North America, including 174 species in the CIS countries [1–3]. Wormwood is widely distributed in various ecological conditions: steppes, deserts, meadows, forests, mountainous areas, some of which are found as weeds in all zones. Wormwoods include annual, biennial, and perennial plants, as well as semi-shrubs and shrubs, reaching heights of 5–170 cm. [4–10] There are 81 species of wormwood growing in Kazakhstan, 17 of which are endemic [4]. After systematizing this data, we began work on analyzing the species composition and distribution of wormwood in our scientific article.

Artemisia L. a great contribution to the study of the genus was made by the scientist V. Besser, who divided the flowers of wormwood into three groups according to their sexual composition: Seriphidium Bess, Dracunculus Bess, Abrotanum Bess. [11]. Scientists who described a significant part of the genus and its species were: C. Linnaeus, A. DeCandolle, Weber, K.F. Ledebour, I.M. Krashennikov [12]. In addition, information about wormwood is found in the works of I. Gmelin, V. Besser, K.F. Ledebour, I.M. Krasnoborov. *Artemisia* L. was traditionally accepted as a relative and was approved by C. Linnaeus [13].

Also I.M. Krasheninnikov [12] made a significant contribution to the systematics of the genus by grouping related species into cycles and series. He described numerous species and provided detailed information on their geographic distribution. In the book *Flora of Kazakhstan*, N. S. Filatova presented a comprehensive description of the genus *Artemisia* L., including its subgenera occurring in Kazakhstan [4].

The classification of the genus Artemisia has undergone significant changes, reflecting progress in botanical science. Early taxonomic systems were based on morphological features, while modern approaches use molecular-phylogenetic data. In the middle of the 20th century, in "Flora of the USSR", P.P. Poliakov [5] divided the genus *Artemisia* into three main groups: *Artemisia* L., *Seriphidium* (Bess.) Rouy, *Dracunculus* (Bess.) Rydb. These groups, in turn, were divided into separate sections. This classification was relevant for its time, and the vast majority of species growing on the territory of Kazakhstan belong to the subgenus Seriphidium. Based on a more detailed morphological analysis, researchers such as Hobbs and Baldwin [14] proposed an expanded classification, dividing the genus *Artemisia* into six subgenera: Absinthium, Artemisia, Dracunculus, *Seriphidium*, Tridentatae, and Pacifica. But in 2023 Jiao B., Chen C., Wei M. et al. [15] after Molecular phylogenetic research proposed a new classification and expansion of the genus Artemisia to 8 subgenera (added subgenera *Pectinata* and *Ponticat*): *Dracunculus, Pectinatae, Pacifica, Ponticae, Seriphidium, Tridentatae, Absinthium, Artemisia*.

The Jetisu region we are studying is located in the southeastern part of our republic and was established as a separate region in 2022 as a result of the division of Almaty region into two regions. Currently, the Jetisu Region borders the Almaty Region to the south, the Karaganda Region to the northwest across Lake Balkhash, the Abai Region to the north, and China to the east. Accordingly, it has a complex ecological structure, from mountain forests and alpine meadows to semi-desert areas. It, in turn, is characterized by a diversity of plants. Systematizing this data, our study analyzes the species composition and distribution of wormwood throughout Jetisu region.

Experimental

In the process of writing this article we study materials from the herbarium fund of the Institute of Botany and Phytointroduction of Almaty city (AA), herbarium of the Al-Farabi Kazakh National University (AFKNU), herbarium of Moscow University (MW) and international platforms such as GBIF, iNaturalist were reviewed to study the species composition and distribution features of species of the genus *Artemisia* L. distributed in the Zhetysu region. In addition, the databases "Flora of Kazakhstan" (1960), "Illustrative Dictionary of Kazakhstan" (1969) and POWO (https://powo.science.kew.org) were used to identify species.

Results and Discussion

According to the system of classification of all flora by P.P. Poliakov [5], the genus *Artemisia* is divided into three genera: *Artemisia* Less., *Dracunculus* (Bess.), Rydberg and *Seriphidium* (Bess.) Rouy. According to The Plant List, there are over 500 species of wormwood in the world. In Kazakhstan, 81 species are found (Tab. 1). 17 species of wormwood found in Kazakhstan are rare endemic species [4, 8, 16].

Table 1

The list of species composition of the genus *Artemisia* L.
(compiled from literary and herbarium data)

№	The name of the species according to the Flora of Kazakhstan	The name of the species in the POWO database	I	II	III	IV
1	*A. absinthium* L. A. albida (AA)	*A. absinthium* L.	+	+		+
2	*A. austriaca* Jacq.	*A. austriaca* Jacq.	+	+	+	+
3	A. *annua* L.	*A. annua* L.	+	+		+
4	*A. albicerata* Krasch.	*A. arenaria* DC.	+	+		
5	*A. aschurbajevii* Wiknl.	*A. aschurbajewii* C.Winkl.	+	+		+
6	*A. arenaria* DC.	*A. arenaria* DC.	+	+		+
7	*A. dracunculus* L.	*A. dracunculus* L.	+	+		+
8	*A.* eranthema Bge.	*A. eranthema* Bunge	+	+	+	+
9	*A. frigida* Willd.	A. *frigida* Willd.	+	+		+
10	*A. gmelini* Web.	*A. gmelinii* Weber ex Stechm.	+	+		+
11	*A. heptapotamica* Poljak	*A. heptapotamica* Poljak	+	+		+
12	*A. juncea* Kar. & Kir.	*A. juncea* Kar. & Kir.	+	+	+	+
13	*A. laciniata* Willd.	*A. laciniata* Willd.	+	+		+
14	*A. leucodes* Schrenk.	A. leucodes Schrenk.	+	+	+	+
15	*A. macrocephala* Jacq.,	*A. macrocephala* Jacquem. ex Besser	+	+		
16	*A. nitrosa* Web.	*A. nitrosa* Weber ex Stechm.	+	+		+
17	*A. pamirica* Winkl.	*A. dracunculus* var. *pamirica* (C. Winkl.)	+	+	+	
18	*A. pauciflora* Web. *A. maikara* (Krasch.) Pavlov (AFKNU)	*A. pauciflora* Weber ex Stechm.	+	+	+	
19	*A. procera* Willd.	*A. abrotanum* L.	+			
20	*A. rupestris* L.	*A. rupestris* L.	+	+	+	+
21	*A. rutifolia* Steph.	*A. rutifolia* Stephan ex Spreng.	+	+	+	+
22	*A. songarica* Schrenk.	*A. songarica* Schrenk ex Fisch. & C.A. Mey.	+	+	+	+
23	*A. scoparia* Waldst. Et Kit.	*A. scoparia* Waldst. & Kit.	+	+	+	+
24	*A. scopaeformis* Ldb.		+	+		

Continuation of Table 1

№	The name of the species according to the Flora of Kazakhstan	The name of the species in the POWO database	I	II	III	IV
25	*A. sieversiana* Willd., Kar.		+	+	+	+
26	*A. santolina* Schrenk.	*A. santolina* Schrenk.	+	+		+
27	*A. semiarida* (Krasch. Et Iljin)	*A. semiarida* (Krasch. & Lavrenko) Filatova	+	+		
28	*A. serotina* Bge. *Seriphidium serotinum* (AA)	*A. oliveriana* J.Gay ex Besser	+	+	+	+
29	*A. sublessingiana* (Kell.) Krasch.	*A. sublessingiana* (B.Keller) Krasch. ex Poljakov	+	+	+	+
30	*A. schrenkiana* Ldb.	*A. schrenkiana* Ledeb.	+	+	+	+
31	*A. marschalliana* Spreng.	*A. marschalliana* Spreng.	+			
33	*A. terrae-albae* Krasch.	*A. terrae-albae* Krasch.	+	+	+	+
34	*A. turanica* Krasch.	*A. turanica* Krasch.	+	+	+	
35	A. *tournefortiana* Rchb.	*A. tournefortiana* Rchb.	+	+		+
36	*A. tomentella* Trautv.	*A. tomentella* Trautv.	+			
38	*A. vulgaris* L.	*A. vulgaris* L.	+	+		+
39	*A. santolinifolia* (AA), *(KazNU)*	*A. stechmanniana* Besser		+	+	+
40	*A. salina* Willd. *(KazNU)* MW	*A. maritima* subsp. *maritima*		+	+	+
41	*Seriphidium kaschgaricum* (AA)	*A. kaschgarica* Krasch.		+		
	Species in total:		38	36	18	28

I — data on the Flora of Kazakhstan, 1960; II — on the Herbarium of the Institute of Botany and Phytointroduction (AA); III — on the Herbarium of the Al-Farabi Kazakh National University (AFKNU), IV — on the Herbarium of Moscow University (MW)

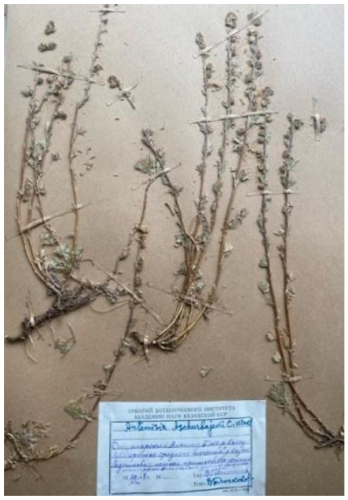
The endemic species of wormwood include the following species: *A. succulenta* Ldb., *A. tomentella* Trautv., *A. albicerata* Krasch., *A. quinqueloba* Trautv., *A. scopaeformis* Lbd., *A. halophile* Krasch., *A. semiarida* (Krasch. Et Lavr.) *A. heptapotamica* Poljak., *A aralensis* Krasch., *A. camelorum* Krasch., *A. amoena* Poljal., *A. transiliensis* Poljak., *A. karatavica* Krasch. et Abol. ex Poljak, *A. mucronulata* Poljak., *A. cina* Berg., *A. valida* Krasch. [4]. However, according to modern sources (https://powo.science.kew.org), the endemic species have been revised, that is, of the above-mentioned species, only *A. quinqueloba* Trautv., *A. aralensis* Krasch., *A. camelorum* Krasch., *A. saissanica* (Krasch.), *A. karatavica* Krasch. et Abol. ex Poljak, *A. mucronulata* Poljak., and *A. cina* Berg. are endemic.

A. cina Berg. (Darmene) is an endemic species of special importance as a medicinal herb and is included in the Red Data Book of Kazakhstan [17]. The aerial part of the plant is rich in essential oils, cyclitols, sesquiterpenoids, flavonoids and nitrogenous compounds. These natural components make the plant an effective anthelmintic, analgesic, anti-inflammatory, anti-tuberculosis, anti-tumor, antibacterial, antifungal, as well as a blood pressure stabilizer [18–20].

According to the floristic zoning of Kazakhstan, as indicated in the Flora of Kazakhstan [4] the *Artemisia* L. species found in the Dzungarian Alatau include *A. laciniata* Willd., *A. gmelinii* Web., *A. tournefortiana* Rchb., *A. rupestris* L., *A. frigida* Willd., *A. aschurbajewii* Wiknl., A. *annua* L., A. *austriaca* Jacq., A. *absinthium* L., A. *sieversiana* Willd., A. *dracunculus* L., *A. rutifolia* Steph., A. *macrocephala* Jacq., *A. pamirica* Winkl., *A. juncea* Kar., *A. heptapotamica* Poljak. And in the Balkhash-Alakulsky floristic district, which is now the territory of the Jetisu region, the wormwood types are found: A. *vulgaris* L., *A. procera* Willd., *A. tournefortiana* Rchb., *A. marschalliana* Spreng., *A. tomentella* Trautv., *A. arenaria* DC., *A. albicerata* Krasch., *A. songarica* Schrenk., *A. eranthema* Bge., *A. scoparia* Waldst. Et Kit., *A. santolina* Schrenk., *A. leucodes* Schrenk., *A. juncea* Kar., *A. scopaeformis* Ldb., *A. annua* L., *A. austriaca* Jacq., *A. absinthium* L., *A. sieversiana* Willd., *A. dracunculus* L., *A. terrae-albae* Krasch., *A. semiarida* (Krasch. Et Iljin), *A. pauciflora* Web., *A. nitrosa* Web., *A. schrenkiana* Ldb., *A. sublessingiana* (Kell.) Krasch., *A. serotina* Bge., *A. turanica* Krasch.

According to literary data, the following species of wormwood are found in the Jetisu region: *A. aschurbajewii, A. sieversiana, A. absinthium, A. heptapotamica, A. sublessingiana, A. frigida, A. scoparia, A. arenaria, A. schrenkiana, A. songarica, A. santolina, A. nitrosa, A. terrae-albae, A. pauciflora, A. vulgaris, A. austriaca, A. juncea, A. gmelinii, A. annua, etc.* [21, 22].

According to the label data in the herbarium collections of the Institute of Botany and Phytointroduction (AA), the following species of Artemisia L. are frequently found in the Zhetysu Alatau (Fig. 1): *A. annua*, *A. absinthium*, *A.* albida, *A.* aschurbaoevii, *A. austriaca*, *A.* juncea, *A.* heptapotamica, *A. frigida*, *A. rutifolia*, *A. rupestris*, *Seriphidium serotinum* (*A. oliveriana*), *A.* serotina, *A.* sublessingiana, *A. sieversiana*, *A.* scoparia, *A.* santolina, *A. santolinifolia*, *A. gmelina*, *A. laciniata*, *A. kaschgarica*, *Seriphidium kaschgaricum* (A. *kaschgarica*). And we observe that the majority of it was collected by N.V. Pavlov in 1928, S. Yu. Lipshits in 1928, N.V. Shipchinsky in 1928, V.P. Goloskokov in 1948, 1956, 1959, 1971, P. Polyakov in 1950, 1953, 1955, 1960 and 1961. Furthermore, the herbarium specimens collected by E.P. Mataeva in 1930, E. Cherniakowska in 1930, N.I. Rubtsov 1934, P. Polyakov, L.A. Kupriyanova, 1934, Godvinsky, 1958, BoranbaevaM.S.1987, T.M. Kudabaeva, N.V. Nelina 1993, 2018, I.M. Krasnoborov 1995, A.N. Kupryanova 2014, Ramazanova 2017, M.P. Danilov et al. 2018, 2019 are also stored there.

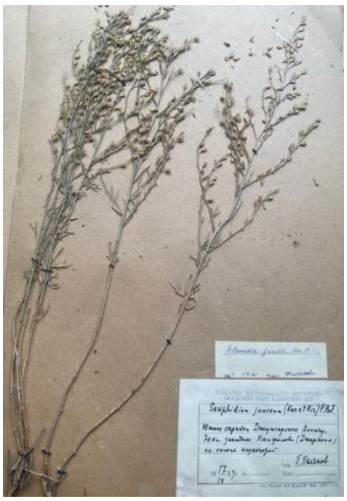


A. aschurbajewii Wiknl

A. austriaca Jacq

A. absinthium L

A. kaschgarica Krasch



A. juncea Kar

A. heptapotamica Poljak

Figure 1. Herbarium specimens of some species of the genus *Artemisia* L. collected in the Zhetysu Alatau (AA)

According to the label information in the Al-Farabi Kazakh National University herbarium fund (AFKNU), herbariums of 73 species of wormwood are preserved, including the following species of wormwood found in the region of Jetisu region: *A. austriaca* Jacq., *A. eranthema* Bge., *A. juncea* Kar. & Kir., *A. leucodes* Schrenk., *A. pamirica* Winkl., *A. maikara* (A. Pauciflora), *A. rupestris* L., *A. rutifolia* Steph., *A. songarica* Schrenk., *A. scoparia* Waldst. & Kit., *A. sieversiana*, *A. serotina* Bge. (*A. oliveriana*), *A. sublessingiana*, *A. schrenkiana*, *A. terrae-albae*, *A. turanica*, *A. santolinifolia* (*A. stechmanniana*), *A. salina* (*A. maritima*). The majority of herbarium specimens collected from the region of Jetisu region were collected by P.P. Polyakov and L.A. Kupriyanova (1934), Ageeva N.T. (1932, 1951, 1969), Karnilova V.S. (1947),

Karnilova V.S., Tkanova (1947), Tkanova (1947), Tarabaeva B.I. (1945, 1946), Krashennikov I.M., Linchevsky I.A. and O.A. We note that Linchesky (1934), collected. In addition, P. Volkova (1931), N.I. Rubtsov, Krivova (1935), Mirovnova (1935), V.P. Goloskokov (1936), Sushkov (1936), Shokolova (1946), Ogai (1946), Solomchenko (1949), Kalekenov (1951), Mambetov (1951), Lashkina M. (1958), Lutsai. (1959), Penkin (1970), Popova (1970), A. Samsonova (1973), Asanova T.T. (1984), Boranbaeva (1987), Aipeisova S. (1987), Aldabekova (1990) herbarium specimens have been preserved.

In the herbarium data of the Moscow University (MW) fund, species belonging to the genus *Artemisia* L. were considered by the Department of Herbarium of Central Asia and Kazakhstan, the herbarium of Muyunkum, Pribalkhashye and Betpak-Dala, Dzhungarsky Alatau and Tarbagatay District. Of these, 153 herbarium data belonging to 29 species were collected from the region that is now part of the Jetisu region. The majority were collected from Semirechye, Taldy-Kurgansky uyezd, Sarkandsky district, Dzhungarsky Alatau, and other nearby regions. Species with coordinates are shown in Table 2 and Figure 2.

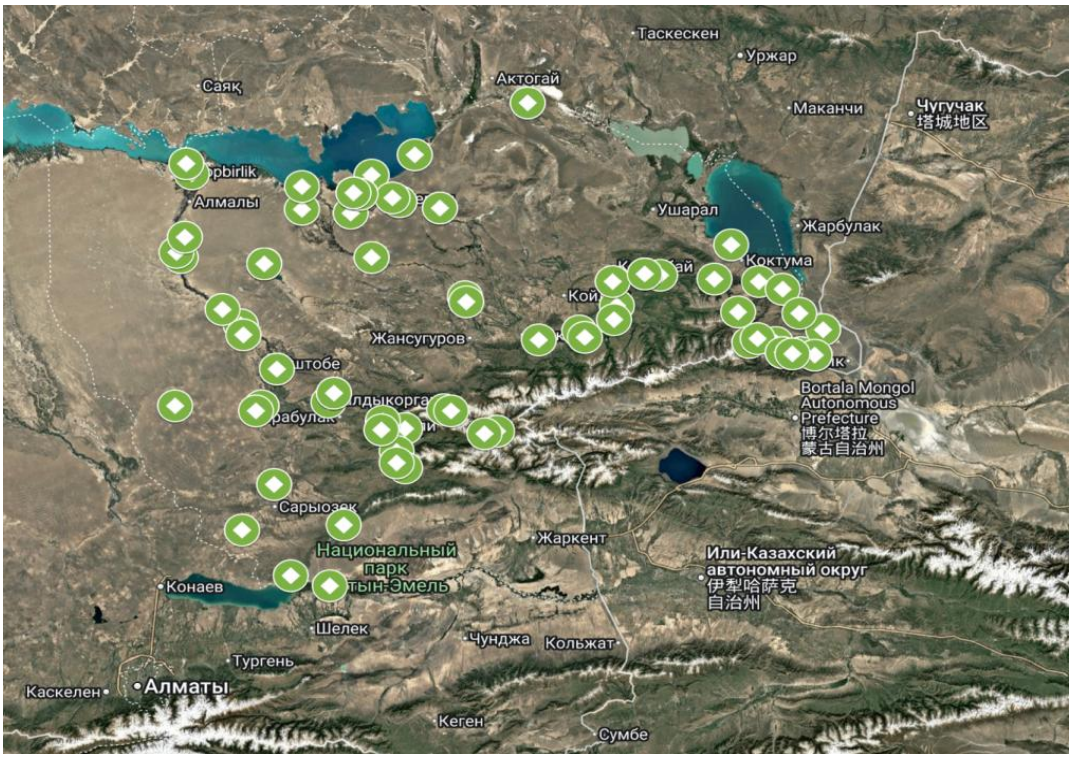


Figure 2. Distribution area of *Artemisia* L. in the Jetisu region

Table 2

Distribution area of the genus *Artemisia* L. in the Jetisu region

№	Species	Coordinates	Dataset
1	*A. absinthium* L.	45°26'00.0"N,80°21'00.0"E	[22]
2		**45°00′16.67″ N,78°22′37.00″ E**	iNaturalist Research-grade Observations
3		44°50′22.79″ N,78°58′38.77″ E	iNaturalist Research-grade Observations
4		44°41′48.61″ N,78°54′57.25″ E	iNaturalist Research-grade Observations
5	A. *annua* L.	45°28'41.1"N077°40'44.7"E	AA
6	*A. arenaria DC.*	46°22′43.45″ N,78°42′48.03″ E	iNaturalist Research-grade Observations
7		46°10'27.4476"N78°10'19.8732"E	MW
8		46°15'47.5380"N78°38'2.2416"E	MW
9		45°50'43.7352"N77°52'24.5892"E	MW
10		45°53'5.0388"N78°42'48.4920"E	MW

Continuation of Table 2

№	Species	Coordinates	Dataset
11		46°15'47.5380"N78°38'2.2416"E	MW
12	*A. arenaria* DC., *A. scoparia* Waldst. Et Kit., *A. terrae-albae* Krasch.	45°19'00.1"N77°19'00.1"E	[22]
13	*A. arenaria* DC., *A. santolina* Schrenk.	44°58'59.9"N77°10'00.1"E	[22]
14	*A. aschurbajewii* C.Winkl.	44°52'59.9"N78°48'00.0"E	[22]
15		44°57′13.24″ N,79°17′23.84″ E	iNaturalist Research-grade Observations
16		45°22'12.6372"N81°41'51.3492"E	MW
17		45°17'23.7228"N82°12'41.5836"E	MW
18		44°49'48.0000"N78°47'24.0000"E	MW
19		45°45'00.0"N81°25'00.1"E	[22]
20	*A. austriaca* Jacq.	45°18'25.4196"N82°05'54.1176"E	MW
21		45°18'11.7612"N81°57'41.1876"E	MW
22	*A. dracunculus* L.	46°16'21.4428"N78°34'8.0292"E	MW
23		46°12'58.6800"N78°56'3.4008"E	MW
24		45°03′40.95″ N,78°25′07.17″ E	iNaturalist Research-grade Observations
25		44°35′44.48″ N,78°59′09.70″ E	iNaturalist Research-grade Observations
26		45°03′40.95″ N, 78°25′07.17″ E	iNaturalist Research-grade Observations
27	*A. frigida* Willd.	45°22'50.3"N80°01'50.4"E	AA
28		45°22'12.6372"N81°41'51.3492"E	MW
29		45°17'23.7228"N82°12'41.5836"E	MW
30		44°14'33.7488"N78°29'55.8924"E	MW
31	*A. frigida* Willd., A. sublessingiana (Kell.) Krasch.	45°24'00.0"N75°51'59.9"E	[22]
33	*A. gmelini* Web.	45°22'0.5880"N81°54'18.1800"E	MW
34		45°17'42.2016"N82°01'39.7272"E	MW
35		45°12'12.3912"N77°57'59.8320"E	MW
36		45°26'28.6224"N82°16'30.8568"E	MW
38	*A. gmelini* Web. *A. vulgaris* L.	45°43'59.9"N81°46'00.1"E	[22]
39	*A. heptapotamica* Poljak	45°33'11.3"N81°36'33.0"E	AA
40		44°13'00.00"N77°42'00.00"E	AA
41		45°35'23.6"N80°38'51.0"E	AA
42	*A. juncea* Kar. & Kir.	44°29'34.0800"N77°56'45.9600"E	MW
43	*A. laciniata* Willd.	45°18'11.7612"N81°57'41.1876"E	MW
44		45°18'25.4196"N82°05'54.1176"E	MW
45	*A. maritima* subsp. *maritima*	45°53'4.8804"N77°12'37.3284"E	MW
46	*A. nitrosa* Weber ex Stechm., A. scoparia Waldst. Et Kit.	45°55'00.1"N77°10'59.9"E	[22]
47	*A. rupestris* L.	45°23'13.92"N81°45'23.4"E	MW
48		45°17'42.2016"N82°01'39.7272"E	MW
49		45°18'11.7612"N81°57'41.1876"E	MW
50		44°49′31.92″ N,79°42′12.14″ E	iNaturalist Research-grade Observations
51	*A. rutifolia* Stephan ex Spreng.	45°46'57.8208"N80°52'15.7692"E	MW

Continuation of Table 2

№	Species	Coordinates	Dataset
52		45°46'31.7460"N80°59'42.5724"E	MW
53		45°47'6.4392"N80°52'14.5344"E	MW
54	*A. santolina* Schrenk.	46°22'59.9"N77°18'00.0"E	[22]
55		46°00'5.3748"N77°14'26.7108"E	MW
56		46°18'30.0348"N78°10'15.5496"E	MW
57		45°50'43.7352"N77°52'24.5892"E	MW
58		46°15'47.5380"N78°38'2.2416"E	MW
59	*A. schrenkiana* Ldb.	43°52'01.2"N78°22'58.8"E	[22]
60		46°10'38.1864"N79°15'4.5036"E	MW
61		46°12'58.6800"N78°56'3.4008"E	MW
62	*A. schrenkiana* Ldb. *A. pauciflora* Weber ex Stechm.	43°55'59.9"N78°28'00.1"E	[22]
63	*A. scoparia* Waldst. Et Kit.	46°09'00.0"N78°33'00.0"E	[22]
64		44°58'59.9"N77°51'00.0"E	[22]
65		46°14′17.60″ N,78°52′50.69″ E	iNaturalist Research-grade Observations
66		46°29′41.98″ N,79°03′45.34″ E	iNaturalist Research-grade Observations
67		46°48′38.74″ N,79°56′58.68″ E	iNaturalist Research-grade Observations
68		45°57′41.28″ N,81°32′59.74″ E	iNaturalist Research-grade Observations
69		45°34′13.97″ N,77°32′23.98″ E	iNaturalist Research-grade Observations
70		45°24′49.92″ N,77°42′24.37″ E	iNaturalist Research-grade Observations
71		45°17'47.8140"N82°02'3.1164"E	MW
72		45°17'23.7228"N82°12'41.5836"E	MW
73		45°38'54.6324"N79°27'6.3036"E	MW
74		46°16'21.4428"N78°34'8.0292"E	MW
75	*A. scoparia* Waldst. Et Kit.	44°57'00.0"N77°48'00.0"E	[22]
76	*A. serotina* Bge.	45°50'43.7352"N77°52'24.592"E	MW
77	*A. sieversiana* Ehrh. ex Willd.	44°37′53.70″ N,78°54′50.83″ E	iNaturalist Research-grade Observations
78		45°44′11.07″ N,80°37′20.61″ E	iNaturalist Research-grade Observations
79		45°26'28.6224"N82°16'30.8568"E	MW
80		45°17'23.7228"N82°12'41.5836"E	MW
81		45°30'36.5868"N80°37'44.8860"E	MW
82	*A. songarica* Schrenk ex Fisch. & C.A. Mey.	46°15'47.5380"N78°38'2.2416"E	MW
83	*A. stechmanniana* Besser	43°56'00.0"N78°05'00.1"E	[22]
84		45°23′33.44″ N,80°24′28.29″ E	iNaturalist Research-grade Observations
85		44°44′38.06″ N,79°13′37.42″ E	iNaturalist Research-grade Observations
86		45°18'11.7612"N81°57'41.1876"E	MW
87	*A. sublessingiana* (Kell.) Krasch.	45°41'19.8744"N81°57'45.5112"E	MW
88		46°10'38.1864"N79°15'4.5036"E	MW
89	*A. terrae-albae* Krasch.	45°32'38.3604"N82°05'25.9080"E	MW
90	*A. tournefortiana* Rchb.	46°16'21.4428"N78°34'8.0292"E	MW
91		45°12'12.3912"N77°57'59.8320"E	MW
92		46°26'33.9576"N77°15'24.1812"E	MW
93		45°36'44.8560"N79°28'0.5988"E	MW
94	*A. viridis* Willd.ex DC.	44°57′0.67″ N,79°20′31.31″ E	iNaturalist Research-grade Observations

Continuation of Table 2

№	Species	Coordinates	Dataset
95		44°48′43.07″ N,79°36′8.73″ E	iNaturalist Research-grade Observations
96	*A. vulgaris* L.	45°47'6.4392"N80°52'14.5344"E	MW
97		45°17'47.8140"N82°02'3.1164"E	MW
98		45°49′5.37″ N,81°04′31.42″ E	iNaturalist Research-grade Observations
99		45°24'00.0"N80°24'00.0"E	[22]

In addition, as a result of field research funded by the Abai Kazakh National Pedagogical University (Tab. 3), the following distribution area of wormwood was identified.

Table 3

Data from species distribution in Abai Kazakh National Pedagogical University

№	Species	Coordinates (N/E)	Height above sea level, m
1	*Artemisia leucodes* Schrenk.	44°58′37.74″; 77°53′58.87″	430
2	*Artemisia nitrosa* Web.	44°52'00.1"; 77°30'00.0"	500
3	*Artemisia terrae-albae* Krasch.	44°55'59.9"; 77°45'00.0"	435
4	*Artemisia absinthium* L.	44°52'19.2"; 78°48'03.6"	1100
5	*Artemisia austriaca* Jacq.	44°52'44.2"; 78°48'10.0"	1350
6	*Artemisia rutifolia* Steph.	44°52'40.9"; 78°48'39.7"	1150

Wormwoods are widely distributed throughout the Jetisu region. Many species of the *Artemisia* L. genus have been widely used in folk medicine for treating tumors, inflammatory, infection, bacterial and viral diseases, as well as illnesses like malaria, and some of these uses are still preserved today [23, 24].

After analyzing scientific articles, we came to the conclusion that the following types of wormwood are currently being actively studied in terms of their chemical composition: *A. vulgaris*, *A. pontica*, *A. annua*, *A. absinthium*, *A.* selengensis, *A. gmelini*, *A. frígida*, *A. mongolica*, *A. jacutica*, *A. argyi*, *A. austriaca*, *A. austrica*, *A. abrotanum*, *A. arctica*, *A. glabella*, *A. gmelinii*, *A. frígida*, *A. sieversiana* and others [25–29].

Types of wormwood used for fodded purposes include *A. pontica*, *A. obtusiloba*, *A. frigida*, *A. marschalliana*, *A. kelleri*, *A. scoparia*, *A. santolina*, *A. halophila*, *A. lercheana*, *A. terrae-albae*, *A. semiarida*, *A. pauciflora*, *A. nitrosa*, *A. schrenkiana*, *A. vulgaris*, A. *austriaca*, *A. lessingiana* and others [4, 30].

In addition to their medicinal uses, wormwoods are also popular as decorative, aromatic, and culinary herbs. *A. dracunculus*, *A. abrotanum*, *A. absinthium*, *A. annua*, *A. frigida*, *A. japonica*, *A. pontica*, A. *scoparia*, *A. vulgaris*, etc. are used for food purposes worldwide [31–35].

Analysis of the studied literature and herbarium materials made it possible to determine the current state of knowledge about the genus *Artemisia* L. The practical significance of this work lies in planning expedition routes and clarifying the distribution areas of the species.

Conclusions

In conclusion, it was found that 81 species of the genus *Artemisia* L. grow in various ecological zones of Kazakhstan, and more than 35 species are distributed in the Jetisu region. In addition, more than 90 GPS coordinates were identified, which allow planning expedition routes. Their value in medicinal, folk medicine, fodder, culinary and perfumery industries was determined. A review and description of the species of the genus *Artemisia* L. distributed in the Zhetysu Alatau and Jetisu region was given, and the need for further field research to develop protection measures was noted.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript: **Seidekhan M.K. —** conceptualization, data analysis, investigation, writing draft; **Aidarbayeva D.K**. — methodology, data curation, data collection.

References

1 Bora K. S. The genus Artemisia: a comprehensive review / K. S. Bora, A. Sharma // Pharmaceutical biology. — 2011. — Vol. 49, No. 1. — P. 101–109. https://doi.org/10.3109/13880209.2010.497815

2 Koul B. The *Artemisia* genus: A review on traditional uses, phytochemical constituents, pharmacological properties and germplasm conservation / B. Koul, P. Taak, A. Kumar // J Glycomics Lipidomics. — 2017. — Vol. 7, No. 1. — P. 142. DOI: 10.4172/2153-0637.1000142

3 Mucciarelli M. Introduction to the genus / M. Mucciarelli, M. Maffei // Artemisia. — Boca Raton: CRC Press, 2001. — P. 16–57.

4 Павлов Н. В. Флора Казахстана: [в 13 т.] / Н. В. Павлов. — Алма-Ата: Академия наук Казахской ССР, Институт ботаники, 1966. — Т. 9. — С. 76–140.

5 Поляков П. П. Род Полынь — *Artemisia* L / П. П. Поляков // Флора СССР. — Москва; Ленинград: Изд-во АН СССР, 1961. — Т. 26. — С. 425–631.

6 Айдарбаева Д. Қ. Растительные ресурсы Казахстана и их рациональное использование: учебное пособие / Д. Қ. Айдарбаева. — Караганда: ИП «Издательство АҚНҰР», 2014. — 194 с.

7 Абдулина С. А. Список сосудистых растений Казахстана / С. А. Абдулина. — Алматы, 1999. — 147 с.

8 Егеубаева Р. А. Дикорастущие эфирномасличные растения юго-востока Казахстана / Р. А. Егеубаева. — Алматы, 2002. — 241 с.

9 Gabdullin E. M. *Artemisia* L. (Subgen. Seriphidium (Bess.) Peterm. in Kazakh Upland / E. M. Gabdullin, A. N. Kupriyanov, S. M. Adekenov // News of the National Academy of Sciences of the Republic of Kazakhstan. Series of Biological and Medical. — 2020. — Vol. 4, No. 340. — P. 46–51. https://doi.org/10.32014/2020.2519-1629.31

10 Дикорастущие полезные растения России / отв. ред. А. Л. Буданцев, Е. Е. Лесиовская. — Санкт-Петербург: Издательство СПХФА, 2001. — С. 74–81.

11 Ишмуратова М. Ю. Биоэкологическое и фитохимическое исследование перспективных видов рода *Artemisia* L.: дис. ... канд. биол. наук / М. Ю. Ишмуратова. — Караганда, 2004. — С. 7–32.

12 Крашенинников И. М. Опыт филогенетического анализа некоторых евроазиатских групп рода *Artemisia* L. в связи с особенностями палеогеографии Евразии / И. М. Крашенинников // Материалы по истории флоры и растительности СССР: сб. ст. — Москва: Изд. АН СССР, 1946. — Вып. 2. — С. 87–194.

13 Коробков А. А. Полыни Северо-Востока СССР / А. А. Коробков. — Л., 1981. — 120 с.

14 Hobbs C. R. Asian origin and upslope migration of Hawaiian *Artemisia* (Compositae–Anthemideae) / C. R. Hobbs, B. G. Baldwin // Journal of Biogeography. — 2013. — Vol. 40. — P. 442–454. DOI: 10.1111/jbi.12046.

15 Jiao B. Phylogenomics and morphological evolution of the mega-diverse genus *Artemisia* (Anthemideae, Asteraceae): implications for its circumscription and infrageneric taxonomy / B. Jiao // Annals of Botany. — 2023. — Vol. 131. — P. 867–883. DOI: 10.1093/aob/mcad051.

16 Иллюстрированный определитель растений Казахстана [в 2-х т.]. — Алма-Ата: Институт ботаники, 1972. — Том 2. — С. 356–373.

17 Қазақстанның қызыл кітабы. — Астана: ЖШС «АртPrintXXI», 2014. — 2-ші том: Өсімдіктер. — 452 б.

18 Rakhymberdieva Zh. S. Molecular genetics plant analysis, *Artemisia* L. Genus, with issr-markers / Zh. S. Rakhymberdieva, A. N. Kaliyeva, G. D. Medeuova // Reports of the National Academy of Sciences of the Republic of Kazakhstan. — 2020. — Vol. 6, No. 334. — P. 35–41. DOI: 10.32014/2020.2518-1483.133.

19 Zhurinov M. An estimation of the antiviral activity and toxicity of biologically active substances obtained from the raw materials of *Artemisia cina* Berg. in vitro and in vivo / M. Zhurinov // Molecules. — 2023. — Vol. 28, No. 14. — P. 5413.

20 Kasmiyati, S. Antibacterial activity and flavonoids content of *Artemisia cina* Berg. ex Poljakov ethyl acetate extracts / S. Kasmiyati // Biosaintifika: Journal of Biology & Biology Education. — 2021. — Vol. 13, No. 1. — P. 106–112. http://dx.doi.org/10.15294/biosaintifika.v13i1.27665

21 Гемеджиева Н. Г. Кадастр ресурсных видов хозяйственно ценных растений Алматинской области / Н. Г. Гемеджиева. — Алматы, 2023. — 180 с.

22 Димеева Л. А. Кадастр растительности Алматинской области / Л. А. Димеева. — Алматы, 2023. — Т. 26. — Вып. 4. — 160 с.

23 Hussain A. The genus *Artemisia* (Asteraceae): a review on its ethnomedicinal prominence and taxonomy with emphasis on foliar anatomy, morphology, and molecular phylogeny: ethnomedicinal prominence and taxonomy of *Artemisia* // Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences. — 2020. — Vol. 57, No. 1. — P. 1–28.

24 Tan R. X. Biologically active substances from the genus *Artemisia* / R. X. Tan, W. F. Zheng, H. Q. Tang // Planta medica. — 1998. — Vol. 64, No. 4. — P. 295–302.

25 Nurlybekova A. Traditional use, phytochemical profiles and pharmacological properties of *Artemisia* genus from Central Asia / A. Nurlybekova // Molecules. — 2022. — Vol. 27, No. 16. — P. 5128. https://doi.org/10.3390/molecules27165128

26 Ишмуратова М. Ю. Интродукция лекарственных растений в центральном Казахстане / М. Ю. Ишмуратова. — Караганда, 2015. — 169 с.

27 Итжанова Х. И. Фармакогностическое изучение полыни гладкой, и разработка лекарственной формы арглабина: автореф. дис. ... канд. фарм. наук / Х. И. Итжанова. — Томск, 2003. — 25 с.

28 Сейдахметова Р. Б. Противовоспалительные свойства эфирного масла *Artemisia glabella* Kar. et Kir. / Р. Б. Сейдахметова // Растительные ресурсы. — 2002. — Т. 38. — Вып. 1. — С. 102–107.

29 Ydyrys A. The systematic assessment of the membrane-stabilizing and antioxidant activities of several kazakhstani plants in the Asteraceae family / A. Ydyrys // Plants. — 2023. — Vol. 13, No. 1. — P. 96. https://doi.org/10.3390/plants13010096

30 Насыров Н. Б. Ақтөбе облысы Әйтеке би ауданы Қайрақты ауылдық округіндегі мал жайылымдарындағы өсімдіктердің мәдени-техникалық жағдайын бағалау / Н. Б. Насыров, М.С. Курманбаева. — Алматы, 2019.

31 Watson L. E. Molecular phylogeny of subtribe Artemisiinae (Asteraceae), including Artemisia and its allied and segregate genera / L. E. Watson et al. // BMC evolutionary Biology. — 2002. — Vol. 2, No. 1. — P. 17.

32 Trendafilova A. Research advances on health effects of edible Artemisia species and some sesquiterpene lactones constituents / A. Trendafilova et al. // Foods. — 2020. — Vol. 10, No. 1. — P. 65. https://doi.org/10.3390/foods10010065

33 Skowyra M. Antioxidant properties of Artemisia annua extracts in model food emulsions / M. Skowyra et al. // Antioxidants. — 2014. — Vol. 3, No. 1. — P. 116–128. https://doi.org/10.3390/antiox3010116

34 Ekiert H. *Artemisia dracunculus* (Tarragon): A review of its traditional uses, phytochemistry and pharmacology / H. Ekiert et al. // Frontiers in Pharmacology. — 2021. — Т. 12. — С. 653993. https://doi.org/10.3389/fphar.2021.653993

35 Zeb S. Pharmacology, taxonomy and phytochemistry of the genus Artemisia specifically from Pakistan: a comprehensive review / S. Zeb et al. // Pharmaceutical and Biomedical Research. — 2019. https://doi.org/10.18502/pbr.v4i4.543

36 POWO. — [Electronic resource]. — Access mode: https://powo.science.kew.org

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Жетісу облысында өсетін *Artemisia* L. туысының көптүрлілігі мен ерекшеліктері

Мақалада әдеби дереккөздер мен гербарий материалдарын талдау негізінде *Artemisia* L. туысының Жетісу облысындағы түрлік құрамы мен таралуы жайлы мәліметтер қарастырылған. *Artemisia* L. Қазақстанның барлық аймақтарында — шөл-шөлейтті, далалы аймақта, таулы жерлерде 81 түрі, Жетісу облысында 35-тен астам түрі кездеседі. Жетісу облысында және Жетісу Алатауында кездесетін түрлеріне кеңейтілген шолу жасалып, зерттеу аймағында кездесетін жусан түрлерінің гербарий деректері (AA, AFKNU, MW гербарий қорлары) берілген. Сонымен қатар гербарий үлгілері мен әдеби деректерді пайдалана отырып Жетісу облысында кездесетін жусанның GPS кординатталары анықталды. Жүргізілген зерттеу *Artemisia* L. туысының таралу аймағы туралы ақпаратты нақтылауға және толықтыруға мүмкіндік береді.

Кілт сөздер: жусан, *Artemisia* L., эндемик, таралуы, гербарий, Қазақстан, Жетісу облысы, Жетісу Алатауы

М.Қ. Сейдехан, Д.К. Айдарбаева

Разнообразие и особенности рода *Artemisia* L., произрастающего в Жетысуской области

В статье представлена информация о видовом составе и распространении рода *Artemisia* L. в Жетысуской области на основе анализа литературных источников и гербарных материалов. 81 вид *Artemisia* L. встречается во всех регионах Казахстана — в пустынной, степной и горной зонах, а более 35 видов — в Жетысуской области. Проведен обширный обзор видов, обнаруженных в Жетысуской области и Жетысуском Алатау, и представлены гербарные данные видов полыни, найденных на исследуемой территории (гербарные фонды AA, AFKNU, MW). Кроме того, с использованием гербарных образцов и литературных данных определены GPS-координаты полыни, найденной в Жетысуской области. Проведенное исследование позволило уточнить и дополнить информацию о распространении рода *Artemisia* L.

Ключевые слова: полынь, *Artemisia* L., эндемик, распространение, гербарий, Казахстан, Жетысуская область, Жетысуский Алатау

References

1 Bora, K. S., & Sharma, A. (2011). The genus Artemisia: a comprehensive review. *Pharmaceutical biology*, *49*(1), 101–109. https://doi.org/10.3109/13880209.2010.497815

2 Koul, B., Taak, P., Kumar, A., Khatri, T., & Sanyal, I. (2017). The *Artemisia* genus: A review on traditional uses, phytochemical constituents, pharmacological properties and germplasm conservation. *J Glycomics Lipidomics*, *7*(1), 142. DOI: 10.4172/2153-0637.1000142

3 Mucciarelli, M., & Maffei, M. (2001). Introduction to the genus. In *Artemisia*, 16–57. CRC Press.

4 Pavlov, N. V. (1966). *Flora Kazakhstana*. Almaty: Akademiia nauk Kazakhskoi SSR, Institut botaniki [in Russian].

5 Poliakov, P. P. (1961). Rod Polyn — *Artemisia* L. [The genus Wormwood — *Artemisia* L.]. *Flora SSSR — Flora of the USSR, 26*, 425–631. Moscow; Leningrad: Izdatelstvo Akademii Nauk SSSR [in Russian].

6 Aidarbayeva, D. K. (2014). *Rastitelnye resursy Kazakhstana i ikh ratsionalnoe ispolzovanie* [Plant resources of Kazakhstan and their rational use]. Karaganda: IP «Izdatelstvo AQŊUR» [in Russian].

7 Abdulina, S. A. (1999). *Spisok sosudistykh rastenii Kazakhstana* [List of vascular plants of Kazakhstan]. Almaty [in Russian].

8 Egeubayeva, R. A. (2002). *Dikorastushchie efirnomaslichnye rasteniia yugo-vostoka Kazakhstana* [Wild essential oil plants of southeastern Kazakhstan]. Almaty [in Russian].

9 Gabdullin, E. M., Kupriyanov, A. N., & Adekenov, S. M. (2020). *Artemisia L*. (Subgen. Seriphidium (Bess.) Peterm. in Kazakh Upland. *News of the National Academy of Sciences of the Republic of Kazakhstan. Series of Biological and Medical, 4*, 340, 46–51. https://doi.org/10.32014/2020.2519-1629.31

10 Budantsev, A. L., & Lesiovskaya, E. E. (Eds.). (2001). *Dikorastushchie poleznye rasteniia Rossii* [Wild beneficial plants of Russia]. Saint Peterburg: Izdatelstvo SPHFA [in Russian].

11 Ishmuratova, M. Yu. (2004). Bioekologicheskoe i fitokhimicheskoe issledovanie perspektivnykh vidov roda *Artemisia* L. [Bioecological and phytochemical study of promising species of the genus *Artemisia* L.]. *Candidate's thesis.* Karaganda (in Russian).

12 Krashennikov, I. M. (1946). Opyt filogeneticheskogo analiza nekotorykh evroaziatskikh grupp roda *Artemisia* L. v sviazi s osobennostiami paleogeografii Evrazii [Experience of phylogenetic analysis of some Eurasian groups of the genus *Artemisia* L. in connection with the peculiarities of paleogeography of Eurasia]. *Materialy po istorii Flory i rastitelnosti SSSR — Materials on the History of the Flora and Vegetation of the USSR*, 2, 87–194. Moscow: Izdatelstvo Akademii Nauk SSSR [in Russian].

13 Korobkov, A. A. (1981). *Polyni Severo-Vostoka SSSR* [Wormwood of the North-East of the USSR]. Leningrad [in Russian].

14 Hobbs, C. R., & Baldwin, B. G. (2013). Asian origin and upslope migration of Hawaiian *Artemisia* (Compositae–Anthemideae). *Journal of Biogeography*, *40*, 442–454. https://doi.org/10.1111/jbi.12046

15 Jiao, B., Chen, C., Wei, M., Chen, S., Sun, S., Zhang, M., & Liu, J. (2023). Phylogenomics and morphological evolution of the mega-diverse genus *Artemisia* (Anthemideae, Asteraceae): implications for its circumscription and infrageneric taxonomy. *Annals of Botany*, *131*, 867–883. https://doi.org/10.1093/aob/mcad051

16 (1972). *Illiustrirovannyi opredelitel rastenii Kazakhstana* [Illustrated determinant of plants of Kazakhstan]. Alma-Ata. Institut botaniki [in Russian].

17 (2014). *Qazaqstannyn qyzyl kitaby* [Red Book of Kazakhstan]. Vols. 1-2. 2-shi tom: *Ösimdikter* — Vol. 2: *Plants*. Astana: ZhShS «ArtPrintXXI» [in Kazakh].

18 Rakhymberdieva, Zh. S., Kaliyeva, A. N., & Medeuova, G. D. (2020). Molecular Genetic Plant Analysis, *Artemisia* L. Genus, With Issr-Markers. *Reports of the National Academy of Sciences of the Republic of Kazakhstan*, *6*(334), 35–41. https://doi.org/10.32014/2020.2518-1483.133

19 Zhurinov, M., Berillo, D., Bazhykova, K. B., Rakhimov, K. D., & Bekezhanova, T. (2023). An estimation of the antiviral activity and toxicity of biologically active substances obtained from the raw materials of Artemisia cina Berg. in vitro and in vivo. *Molecules*, *28*(14), 5413.

20 Kasmiyati, S., Kristiani, E. B. E., Herawati, M. M., & Sukmana, A. B. A. (2021). Antibacterial activity and flavonoids content of Artemisia cina Berg. ex Poljakov ethyl acetate extracts. *Biosaintifika: Journal of Biology & Biology Education*, *13*(1), 106–112. http://dx.doi.org/10.15294/biosaintifika.v13i1.27665

21 Gemedzhieva N. G. (2023). *Kadastr resursnykh vidov khoziaistvenno tsennykh rastenii Almatinskoi oblasti* [Cadastre of resource species of economically valuable plants of the Almaty region]. Almaty [in Russian].

22 Dimeyeva, L. A. (2023). *Kadastr rastitelnosti Almatinskoi oblasti* [Cadastre of vegetation of Almaty region]. Almaty [in Russian].

23 Hussain, A. (2020). The genus artemisia (Asteraceae): a review on its ethnomedicinal prominence and taxonomy with emphasis on foliar anatomy, morphology, and molecular phylogeny: ethnomedicinal prominence and taxonomy of *Artemisia*. *Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences*, *57*(1), 1–28.

24 Tan, R. X., Zheng, W. F., & Tang, H. Q. (1998). Biologically active substances from the genus Artemisia. *Planta medica*, *64*(4), 295–302.

25 Nurlybekova, A., Kudaibergen, A., Kazymbetova, A., Amangeldi, M., Baiseitova, A., Ospanov, M., & Jenis, J. (2022). Traditional use, phytochemical profiles and pharmacological properties of Artemisia genus from Central Asia. *Molecules*, *27*(16), 5128. https://doi.org/10.3390/molecules27165128

26 Ishmuratova, M. Yu. (2015). *Introduktsiia lekarstvennykh rastenii v tsentralnom Kazakhstane* [Introduction of medicinal plants in Central Kazakhstan]. Karaganda [in Russian].

27 Itzhanova, Kh. I. (2003). Farmakognosticheskoe izuchenie polyni gladkoi, i razrabotka lekarstvennoi formy arglabina [Pharmacognostic study of *Artemisia glabella* and the development of the arglabin medicinal form]. *Extended abstract of candidate's thesis.* Tomsk [in Russian].

28 Seydakhmetova, R. B., Pak, R. N., Baisenbayeva, A. A., Rakhimov, K. D., & Adekenov, S. M. (2002). Protivovospalitelnye svoistva efirnogo masla *Artemisia glabella* Kar. et Kir. [Anti-inflammatory properties of the essential oil of *Artemisia glabella* Kar. et Kir.]. *Rastitelnye resursy — Plant resources, 38*(1), 102–107 [in Russian].

29 Ydyrys, A., Zhamanbayeva, G., Zhaparkulova, N., Aralbaeva, A., Askerbay, G., Kenzheyeva, Z., & Murzakhmetova, M. (2023). The systematic assessment of the membrane-stabilizing and antioxidant activities of several kazakhstani plants in the Asteraceae family. *Plants*, *13*(1), 96. https://doi.org/10.3390/plants13010096

30 Nasyrov, N. B., & Kurmanbayeva, M. S. (2019). *Aqtöbe oblysy Äyteke bï audany Qayraqty auyldyq okrugindegi mal zhayylymdaryndağy ösimdikterdiñ mädeni-tekhnïkalyq zhag'dayyn bağalau* [Assessment of the cultural and technical condition of plants in the pastures of the Qairaqty rural district, Äyteke Bı district, Aktobe region]. Almaty [in Kazakh].

31 Watson, L. E., Bates, P. L., Evans, T. M., Unwin, M. M., & Estes, J. R. (2002). Molecular phylogeny of subtribe Artemisiinae (Asteraceae), including Artemisia and its allied and segregate genera. *BMC evolutionary Biology*, *2*(1), 17.

32 Trendafilova, A., Moujir, L. M., Sousa, P. M., & Seca, A. M. (2020). Research advances on health effects of edible Artemisia species and some sesquiterpene lactones constituents. *Foods*, *10*(1), 65. https://doi.org/10.3390/foods10010065

33 Skowyra, M., Gallego, M. G., Segovia, F., & Almajano, M. P. (2014). Antioxidant properties of Artemisia annua extracts in model food emulsions. *Antioxidants*, *3*(1), 116–128. https://doi.org/10.3390/antiox3010116

34 Ekiert, H., Świątkowska, J., Knut, E., Klin, P., Rzepiela, A., Tomczyk, M., & Szopa, A. (2021). Artemisia dracunculus (Tarragon): A review of its traditional uses, phytochemistry and pharmacology. *Frontiers in Pharmacology*, *12*, 653993. doi.org/10.3389/fphar.2021.653993

35 Zeb, S., Ali, A., Zaman, W., Zeb, S., Ali, S., Ullah, F., & Shakoor, A. (2019). Pharmacology, taxonomy and phytochemistry of the genus Artemisia specifically from Pakistan: a comprehensive review. *Pharmaceutical and Biomedical Research*. https://doi.org/10.18502/pbr.v4i4.543

36 POWO. Retrieved from https://powo.science.kew.org.

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Research Article

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Assessment of the cytotoxic activity of humates produced by "Shubarkol Komir" JSC

The development of new humic preparations from locally available raw materials is important for advancing the domestic industry of the Republic of Kazakhstan. In Central Kazakhstan, humic preparations such as potassium humate and sodium humate have been developed by "Shubarkol Komir" JSC based on weathered coal. To confirm their applicability, studies assessing their biological activity and safety are required. The purpose of this study is to evaluate the cytotoxic activity of potassium humate and a mixture of potassium humate and humate in different dilutions. The study materials included humates produced by "Shubarkol Komir" JSC, which were compared with a commercial preparation, ECO humate, using tests on *Saccharomyces cerevisiae* cells and *Artemia salina* nauplii. The test results showed that high concentrations of humic preparations (4 %) had an inhibitory effect on the foaming ability of yeast, which was lower than that of the comparison drug nystatin. Concentrations from 0.5 to 0.005 % did not inhibit *Saccharomyces cerevisiae* and activated the foaming ability. The results of the second experimental series demonstrated that both the humates produced by "Shubarkol Komir" JSC and the reference preparation were non-cytotoxic to *Artemia salina* larvae at all tested concentrations (0.5–0.005%), indicating their safety. Thus, the obtained data confirm the safety of using potassium humate and a mixture of potassium humate and sodium humate produced by "Shubarkol Komir" JSC in agriculture.

Keywords: humic preparations, potassium humate, sodium humate, cytotoxic activity, concentration, safety.

Introduction

The resources and effectiveness of organic fertilizers can be significantly increased through the use of humic fertilizers produced from various natural organic sources (peat, highly oxidized low-ash hard coal, brown coal, and oxidized brown coal, sapropel, organic industrial waste, etc.) containing large amounts of humic acids [1–4].

Humic preparations are complex biologically active substances containing humic acids and humates [5, 6], a balanced set of macro- and microelements, and microflora, which enhance the protective properties of plants and seedlings against a number of fungal and bacterial diseases [7–10].

It should be emphasized that humates are not a source of mineral nutrition and do not replace it, but only increase the coefficient of its utilization, therefore humate solutions can be used together with fertilizers, herbicides, and fungicides [11]. Thus, the use of humates in combination with mineral fertilizers and pesticides not only increases the yield of field crops and their efficiency, but also improves the quality of agricultural products [12].

There is also the problem of effective assimilation of mineral fertilizers, which is central to crop production. The complexity of solving this problem lays in the fact that potassium and nitrogen fertilizers, which are easily soluble in water, are easily washed out of the soil, while phosphorus fertilizers, on the contrary, are bound by calcium, magnesium, aluminum, and iron ions present in the soil into an inert form that is inaccessible to plants [13]. Only in the presence of humic substances does the efficiency of plant assimilation of all nutrients increase dramatically [14].

Humic preparations play an important role in increasing the biological activity of the soil, enriching seeds with macro- and microelements, and as growth stimulants [15]. Of particular interest are complex humic fertilizers, which have been used in agricultural production for a number of years.

The purpose of this study is to evaluate the cytotoxic activity of potassium humate, a mixture of potassium humate and sodium humate, and to determine safe concentrations for use on plants.

Experimental

The objects of the study were humate concentrates (4 %) provided by "Shubarkol Komir" JSC: potassium humate, a mixture of potassium humate and sodium humate (in a ratio of 2:1, respectively). Distilled water and a commercial sample of ESO humates served as controls in the studies. The tested humates were diluted to concentrations of 0.1 %, 0.01 %, 0.5 %, 0.05 %, and 0.005 %. Commercial ESO humate (manufactured by NTO EcoTek, Russia) was used according to the attached instructions — 0.1 %.

Assessment of the cytotoxic activity of humates

The test for cytotoxic activity of humates was carried out in two variants: on a culture of *Saccharomyces cerevisiae* cells and on larvae (nauplii) of *Artemia salina* crustaceans.

The first test consisted of assessing the rate of foam rise in a suspension of *Saccharomyces cerevisiae* yeast [16]. Dry active Pakmaya yeast (Turkey) was used. The antifungal drug Nystatin at a concentration of 5 mg/ml was used as a positive control sample. The drug was first ground in a mortar until a homogeneous mass was obtained, and then dissolved in water. A 10 % DMSO solution was used as a negative control.

Dough balls were prepared as follows: 0.2 g of glucose and 0.68 g of yeast were added to 9 ml of water. 1 ml of the test solution (samples of humates in different dilutions, DMSO, nystatin) was added to the resulting mixture. Three ml of the analyzed sample was transferred to measuring tubes, repeated three times for each tested sample (Fig. 1), and incubated in a thermostat at a temperature of 30ºC for 15 minutes.

After the time had elapsed, the volume of the foam formed and the rate of its rise were determined using the following formula:

$$v = V/t, \quad (1)$$

where v is the foam rise rate (ml/min); V is the foam volume (ml); t is the time (min).

An increase in foam rise velocity was considered a stimulating effect, and a decrease was considered an inhibiting effect compared to the control.

Figure 1. Experiments to study the lifting force of yeast in experiments with humic preparations

Artemia salina nauplii (Fig. 2) were used to conduct the second test for cytotoxic activity [17]. Artificial seawater was prepared for the crustacean culture (Tab. 1).

Table 1

Seawater composition

Component	Molecular weight	Added mass, g
NaCl	58.44	23.926
$Na_2 SO_4$	142.04	4.008
KCl	74.56	0.677

Continuation of Table 1

Component	Molecular weight	Added mass, g
Sodium bicarbonate ($NaHCO_3$)	84.00	0.196
Potassium bromide (KBr)	119.01	0.098
$H_3 BO_3$ (boric acid)	61.83	0.02
Sodium fluoride	41.99	0.003

To conduct the experiments, 200 mg of *Artemia salina* eggs were weighed and placed in 1 liter of artificial seawater. The eggs were kept aerated, under constant lighting and at a temperature of 25 °C for 2-3 days, until the larvae hatched (Fig. 2). After hatching, 20–40 larvae were collected using a Pasteur pipette and placed in a 2 ml cell of a laboratory plate. The number of live and dead larvae in each cell was counted. Ten micrometers of test solutions, positive and negative controls were added to the cells. The number of dead individuals was counted after 1, 4, and 24 hours. The mortality rate (P) was analyzed using the following formula:

$$P = (A — N — B) *100 \%/Z, \quad (2)$$

where A is the number of dead larvae after 24 hours; N is the number of dead larvae before the experiment; B is the average number of dead larvae in the control sample; Z is the total number of larvae.

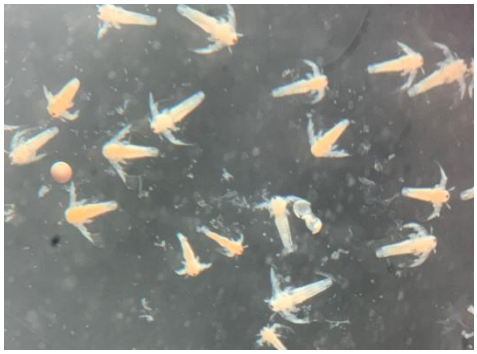

Figure 2. Nauplii of *Artemia salina*

The cytotoxic activity of humates was determined based on the number of dead larvae.

Statistical processing of data on cytotoxic activity was performed using ANOVA dispersion analysis with multiple comparisons according to Dunnet in the GraphPadPrizm 8.0 program.

Results and Discussion

The results of the assessment of the effect of humates on the buoyancy of *Saccharomyces cereviseae* showed that a mixture of sodium/potassium humates in a high concentration (4 %), had a significant inhibitory effect on the foaming activity *of Saccharomyces cereviseae*, which was significantly lower than the control values and the data obtained with the antifungal drug Nystatin. Concentrations ranging from 0.05 to 0.005 % showed a weak stimulating effect, which was significantly higher than the foam rise when using Nystatin, but significantly lower than the results obtained in the control. However, at concentrations of 0.1 % and 0.5 %, on the contrary, the mixture of humates has a significantly stimulating effect on yeast cells (Fig. 3).

When testing potassium humate, it was found that the concentrate of this preparation, like the mixture of humates, has an inhibitory effect on the foaming activity *of Saccharomyces cereviseae*. The foam rise indicators were significantly lower than the control and the indicators obtained when adding the preparation Nystatin. A concentration of 0.5 % potassium humate has a stimulating effect on yeast cells, while a further decrease in concentration shows no stimulating or inhibiting effect (Fig. 4).

Testing of the comparison preparation (humate ESO) also showed that its concentrate (10 %) has neither an inhibitory nor a stimulating effect on the foaming activity *of Saccharomyces cereviseae*. However, a decrease in concentration leads to inhibition of the foaming activity of yeast cells (Fig. 5).

Thus, statistical calculations showed that high concentrations of humic preparations produced by “Shubarkol Komir” JSC (4 %) had a depressing effect on the foaming ability of yeast, which was lower than that of the comparison preparation, the antifungal preparation nystatin. The humic preparation ESO at a con-

centration of 10 % did not show an inhibitory effect. However, humate concentrations of 4–10 % are not used in agriculture, as dilutions of at least 0.1-0.5 % are required.

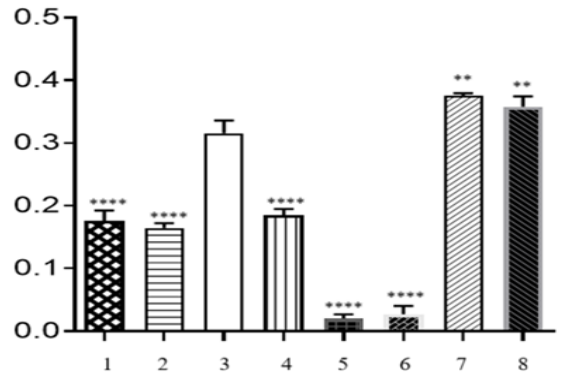

Figure 3. Effect of sodium/potassium humate mixture on the foaming activity *of Saccharomyces cereviseae*: one-way analysis of variance with multiple comparisons by Dunnet; ns — insignificant; **p>0.01; ****p>0.0001. The results are presented as mean ± standard deviation. Vertical axis — foam rise rate, ml/min; experimental variants: 1 — 0.005 %, 2 — 0.01 %, 3 — control, 4 — 0.05 %, 5 — 4 %, 6 — nystatin, 7 — 0.1 %, 8 — 0.5 %

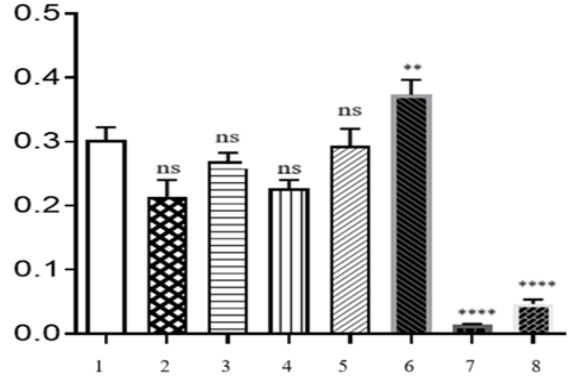

Figure 4. Effect of potassium humate on the foaming activity *of Saccharomyces cereviseae*: one-factor analysis of variance with multiple comparisons by Dunnet; ns — insignificant; **p>0.01; ****p>0.0001. Results are presented as mean ± standard deviation. Vertical axis — foam rise rate, ml/min; experimental variants: 1 — control, 2 — 0.005 %, 3 — 0.01 %, 4 — 0.05 %, 5 — 0.1 %, 6 — 0.5 %, 7 — 4 %, 8 — nystatin

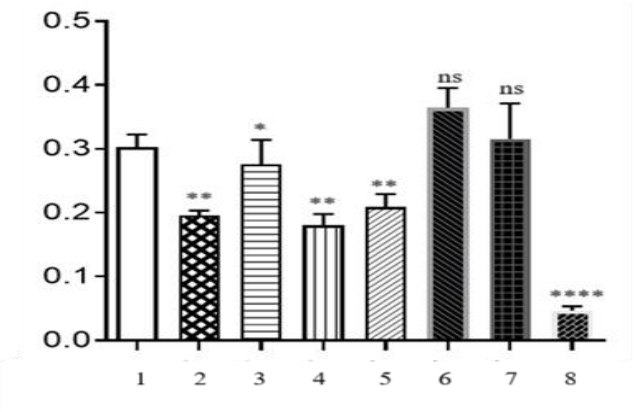

Figure 5. Effect of ESO humate on the foaming activity *of Saccharomyces cereviseae*: one-way analysis of variance with multiple comparisons by Dunnet; ns — insignificant;*p>0.05; **p>0.01; ****p>0.0001. The results are presented as mean ± standard deviation. Vertical axis — foam rise rate, ml/min; experimental variants: 1 — control, 2 — 0.005 %, 3 — 0.01 %, 4 — 0.05 %, 5 — 0.1 %, 6 — 0.5 %, 7 — 10 %, 8 — nystatin

Testing on *Artemia salina* brine shrimp at concentrations ranging from 0.5 to 0.005 % showed no inhibitory effect. According to the data obtained, mortality in samples treated with potassium humate at concentrations of 4 % and 0.5 % significantly exceeds that of the control sample. In addition, the difference in mortality between the control sample and the sample treated with humate at a concentration of 0.05 % is also statistically significant. However, despite this, all tested concentrations of potassium humate produced by "Shubarkol Komir" JSC are not cytotoxic to *Artemia salina* crustaceans, since the mortality of larval nauplii does not exceed 50 % (Fig. 6).

In experiments testing a mixture of potassium and sodium humate, mortality in samples treated with humate at concentrations of 4 % and 0.5 % significantly exceeded that of the control sample. In addition, the difference in mortality between the control sample and the sample treated with a mixture of potassium and sodium humates at a concentration of 0.05 % is also statistically significant. The mortality rate in the sample treated with a 4 % mixture of humates averages 72.2 %, which is much higher than 50 %, indicating that this

concentration is cytotoxic to *Artemia salina* larvae. However, as the concentration of the potassium and sodium humate mixture decreases, cytotoxicity decreases (Fig. 7).

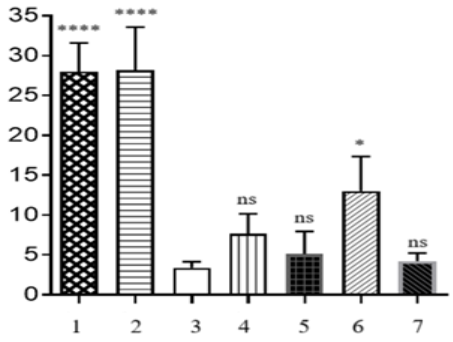

Figure 6. Effect of potassium humate produced by "Shubarkol Komir" JSC on the mortality of *Artemia salina* larvae: one-way analysis of variance with multiple comparisons by Dunnet; ns — insignificant; *p>0.05; ****p>0.0001. The results are presented as mean ± standard deviation. Vertical axis — larval mortality; experimental variants: 1 — 4 %, 2 — 0.5 %, 3 — control, 4 — 0.1 %, 5 — 0.005 %, 6 — 0.05 %, 7 — 0.01 %

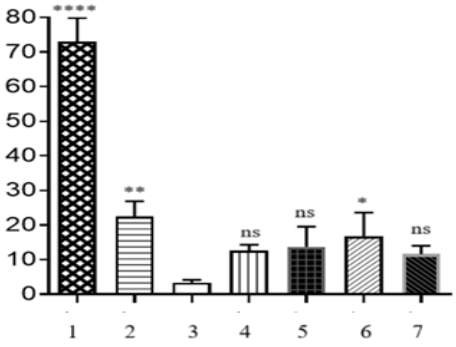

Figure 7. Effect of potassium and sodium humate mixture on *Artemia salina* larval mortality: one-way analysis of variance with Dunnet's multiple comparisons; ns — not significant; *p>0.05; **p>0.01; ****p>0.0001. Results are presented as mean ± standard deviation. Vertical axis — larval mortality; experimental variants: 1 — 4 %, 2 — 0.5 %, 3 — control, 4 — 0.1 %, 5 — 0.005 %, 6 — 0.05 %, 7 — 0.01 %

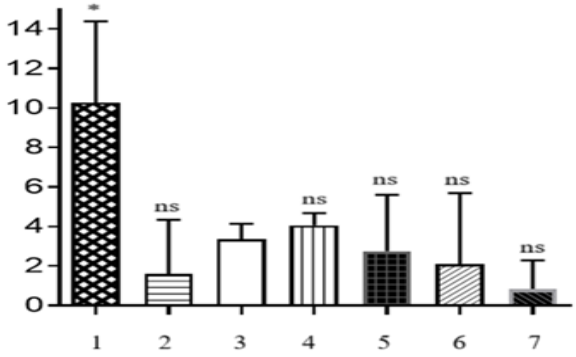

Figure 8. Effect of ECO humate on the mortality of *Artemia salina* larvae: one-way analysis of variance with multiple comparisons by Dunnet; ns — insignificant; *p>0.05. Results are presented as mean ± standard deviation. Vertical axis — larval mortality; experimental variants: 1 — 4 %, 2 — 0.5 %, 3 — control, 4 — 0.1 %, 5 — 0.005 %, 6 — 0.05 %, 7 — 0.01 %

According to the data obtained, the mortality in samples treated with ECO humate at a concentration of 10 % exceeds that of the control sample, but averages 10.2 %, indicating no cytotoxicity towards *Artemia salina* larvae. The results obtained in samples with all tested concentrations indicate that ECO humate is not cytotoxic (Fig. 8).

The data obtained allow us to conclude that humates produced by "Shubarkol Komir" JSC (potassium humate, a mixture of potassium and sodium humates) and the reference preparation (humate ESO) are not cytotoxic to *Artemia salina* larvae at all tested concentrations (from 0.5 to 0.005 %), i.e., the tested concentrations are safe for use.

Conclusion

The assessment of the toxicity of humic preparations on *Saccharomyces cereviseae* cells and *Artemia salina* larvae showed that all humates produced by "Shubarkol Komir" JSC are non-toxic, i.e., safe for use when diluted to concentrations of 0.5 % and below. All studied concentrations of potassium humate, mixtures of potassium and sodium humate, as well as the comparison preparation ESO, can be used in agriculture for plant treatment.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

The manuscript was written with contributions from all authors. All authors have approved the final version of the manuscript: **Zhumina A.G. —** conceptualization, investigation, writing draft; **Orazbay A.D. —** research, methodology; **Martynova Y.N. — investigation, statistical analysis; Abyurov A.Zh. — data curation, analysis; Safronova I.A. —** data collection**; Kulandin M.P., Jexembayev D.M. — humate production.**

References

1 Lobartini J. C. The geochemical nature and agricultural importance of commercial humic matter / J. C. Lobartini, K. H. Tan, J. A. Rema, A. R. Gingle, C. Pape, D. S. Himmelsbach // Science of the total environment. — 1992. — Vol. 113 (1-2). — P. 1–15. https://doi.org/10.1016/0048-9697(92)90013-1

2 Wang R. Production of artificial humic acid from rice straw for fertilizer production and soil improvement / R. Wang, D. Li, F. Deng, Zh. Cao, G. Zheng // Science of total environment. — 2024. — Vol. 906(1). — P. 167548. https://doi.org/10.1016/j.scitotenv.2023.167548

3 Rose M. T. A meta-analysis and review of plant-growth response to humic substances / M. T. Rosa, A. F. Patti, K. R. Little, A. L. Brown, W. R. Jackson, T. R. Cavagnaro // In Advances in Agronomy. — Amsterdam: Elsevier, 2014. — P. 37–89. https://doi.org/10.1016/B978-0-12-800138-7.00002-4

4 Li Y. Humic acid fertilizer improves soil properties and soil microbial diversity of continuous cropping peanut: a three-year experiment / Y. Li, F. Fang, J. Wei, X. Wu, R. Cui, G. Li, F. Zheng, D. Tan // Scientific Report. — 2019. — Vol. 9. — P. 1–9. https://doi.org/10.1038/s41598-019-48620-46

5 Arif M. Humic acids as a feed addictive in poultry diets: a review / M. Arif, M. Alagawany, M. E. Abd El-Hack, M. Saeed, M. A. Arain, S. S. Elnesr // Iran J Vet Res. — 2019. — Vol. 20(3). — P. 167–172.

6 Savelyeva A. V. Detoxication of oil-contaminated soils by using humic acids / A. V. Savelyeva, E. V. Linkevich, N. V. Yudina, N. Nebogina // IOP Conference. Series Materials Science and Engineering. — 2019. — Vol. 597(1). — P. 012020. https://doi.org/10.1088/1757-899X/597/1/012020

7 Tang W. W. Impact of humic/fulvic acid on the removal of heave metals from aqueous solutions using nanomaterials: a review / W. W. Tang, G.-M. Zeng, J. -L. Gong, J. Liang, P. Xu, Ch. Zhang, B.-B. Huang // Sci Total Inviron. — 2014. — Vol. 15(468-469). — P. 1014–1027. https://doi.org/10.1016/j.scitotenv.2013.09.044

8 Clapp C. E. Plant growth promoting activity of HS // Understanding and Managing Organic Matter in Soils, Sediments, and Waters / C. E. Clapp, Y. Chen, M. H. B. Hayes, H. H. Cheng. — Madison: IHSS, 2001. — P. 243–255.

9 Kovalevski D. V. A new approach to description of structure of HS and its application to estimating a quantitative structure — property relationship / D. V. Kovalevski, I. V. Perminova, A. V. Kudryavtsev, V. S. Petrosyan // The role of humic substance in the ecosystem and in environmental protection: Trans. 8th Meeting of IHSS. — Wroclav (Poland), 1996. — P. 99.

10 Riede U. N. Humate induced activation of human granulocytes / U. N. Riede, G. Zeck-Kapp, N. Freudenberg et al. // Virchows Arch. B. Cell Pathol. Incl. Mol. Pathol. — 1991. — Vol. 60, No. 1. — P. 27–34.

11 Горовая А. И. Гуминовые вещества: Строение, функции, механизм действия, протектор, свойства, экологическая роль / А. И. Горовая, Д. С. Орлов, О. В. Щербенко // Нац. акад. Наук Украины, Ин-т проблем природопользования и экологии. — Киев: Наукова думка, 1995. — 303 с.

12 Имбаева Д. С. Влияние биопрепарата на основе гумата калия, обогащенного микроэлементами, на организм лабораторных животных / Д. С. Имбаева, Е. В. Кухар // Материалы Международной научно-теоретической конференции «Сейфуллинские чтения — 16: Молодежная наука новой формации — будущее Казахстана». — 2020. — Т. 1. — Ч.3 — С. 243–245.

13 Карпухин А. И. Функциональная роль комплексных соединений в генезисе почв и питании растений // В сб.: Гуминовые вещества в биосфере / А. И. Карпухин. — М.: Наука, 1993. — С. 117–126.

14 Пироговская Г. В. Использование гуминовых препаратов в получении минеральных удобрений / Г. В. Пироговская, И. А. Богомаз, Е. И. Шагиева, М. Ф. Коваленок, С. И. Кулешова, Г. В. Наумова, Г. И. Райцина // В сб.: Гуминовые вещества в биосфере. — М.: Наука, 1993. — С. 166–174.

15 Abutova R. J. Humic preparation and plant activity / R. J. Abutova, M. K. Kozhakhmetov // Journal of water and land development. — 2022. — Vol. 55. — P. 104–108. https://doi.org/10.24452/jwld.2022.142311

16 Жданова Г. О. Использование *Saccharomyces cerevisiae* для оценки биологической активности лекарственных препаратов / Г. О. Жданова, О. Ф. Вятчина, В. А. Быбин, Д. И. Стом, Г. М. Федосеева // Сибирский медицинский журнал. — 2013. —№ 4. — С. 104–106.

17 Meyer B. N. Brine shrimp: A convenient general bioassay for active plant constituents / B. N. Meyer, N. R. Ferrighi, J. E. Putnam, L. B. Jacobsen, D. E. Nichols, J. L. McLaughlin // Planta Medica. — 1982. — Vol. 45. — P. 31–34.

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«Шұбаркөл Көмір» АҚ өндірген гуматтардың цитотоксикалық белсенділігін бағалау

Жергілікті қолжетімді шикізаттан жаңа гуминдік препараттарды әзірлеу және жасау Қазақстан Республикасының отандық өнеркәсібін дамыту үшін өзекті. Орталық Қазақстанда «Шұбаркөл Көмір» АҚ-ның морыған көмірі негізінде калий гуматы және натрий гуматы сияқты гуминдік препараттар әзірленді. Оларды қолдану мүмкіндігін растау үшін биологиялық белсенділік пен қауіпсіздікті бағалау бойынша зерттеулер қажет. Зерттеудің мақсаты — әртүрлі сұйылтулардағы калий гуматының және калий гуматы мен гумат қоспасының цитотоксикалық белсенділігін бағалау. Зерттеулер «Шұбаркөл Көмір» АҚ өндірген гуматтарда Есо коммерциялық гуматымен салыстырғанда *Saccharomyces cerevisiae* жасушалары мен *Artemia salina* науплий шаян тәрізділерін пайдалана отырып жүргізілген сынақтарда жүргізілді. Тестілеу нәтижелері көрсеткендей, өндірілген гуминді препараттардың жоғары концентрациясы (4 %) ашытқылардың көбіктену қабілетіне тежегіш әсерін көрсетті, бұл нистатинді салыстыру көрсеткіштерінен төмен болды. 0,5-тен 0,005 %-ға дейінгі концентрациялар *Saccharomyces cerevisiae* депрессиясын көрсетпеді және көбіктену қабілетін белсендірді. Тәжірибелердің екінші сериясының нәтижелері «Шұбаркөл Көмір» АҚ өндірген гуматтардың және салыстыру препаратының барлық сыналған концентрацияларында (0,5-тен 0,005 %-ға дейін) *Artemia salina* шаян тәрізділердің дернәсілдеріне қатысты цитоуыттылығы жоқ екенін көрсетті, яғни сыналатын концентрациялар қолдану үшін қауіпсіз. Осылайша, алынған деректер «Шұбаркөл Көмір» АҚ ауыл шаруашылығында калий гуматын және калий гуматы мен натрий гуматының қоспасын қолданудың қауіпсіздігін растайды.

Кілт сөздер: гуминдік препараттар, калий гуматы, натрий гуматы, цитотоксикалық белсенділік, концентрация, қауіпсіздік

А.Г. Жумина, А.Д. Оразбай, Е.Н. Мартынова, А.Ж. Абюров, И.А. Сафронова, М.П. Куландин, Д.М. Жексембаев

Оценка цитотоксической активности гуматов производства АО «Шубарколь комир»

Разработка и создание новых гуминовых препаратов из местного доступного сырья является актуальным для развития отечественной промышленности Республики Казахстан. В Центральном Казахстане на основе выветрелых углей АО «Шубарколь комир» разработаны гуминовые препараты, такие как гумат калия и гумат натрия. Для подтверждения возможности их применения необходимы исследования по оценке биологической активности и безопасности. Цель настоящего исследования — оценить цитотоксическую активность гумата калия и смеси гумата калия и гумата натрия в разных разведениях. Исследования проводили на гуматах производства АО «Шубарколь комир» в сравнении с коммерческим гуматом ECO с использованием тестов на клетках *Saccharomyces cerevisiae* и науплий рачков *Artemia salina.* Результаты тестирования показали, что высокие концентрации гуминовых препаратов производства (4 %) показали угнетающее действие на пенообразовательную способность дрожжей, что было ниже показателей препарата сравнения — нистатином. Концентрации от 0,5 до 0,005 % не показали угнетения *Saccharomyces cerevisiae* и активировали пенообразовательную способность. Результаты второй серии опытов показали, что гуматы производства АО «Шубарколь комир» и препарат сравнения не обладают цитотоксичностью по отношению к личинкам рачков *Artemia salina* во всех протестированных концентрациях (от 0,5 до 0,005 %), то есть испытуемые концентрации являются безопасными для применения. Таким образом, полученные данные подтверждают безопасность применения гумата калия и смеси гумата калия и гумата натрия производства АО «Шубарколь комир» в сельском хозяйстве.

Ключевые слова: гуминовые препараты, гумат калия, гумат натрия, цитотоксическая активность, концентрация, безопасность

References

1 Lobartini, J. C., Tan, K. H., Rema, J. A., Gingle, A. R., Pape, C., & Himmelsbach, D. S. (1992). The geochemical nature and agricultural importance of commercial humic matter. *Science of the total environment., 113* (1-2), 1–15. https://doi.org/10.1016/0048-9697(92)90013-1

2 Wang, R., Li, D., Deng, F., Cao, Zh., & Zheng, G. (2024). Production of artificial humic acid from rice straw for fertilizer production and soil improvement. *Science of total environment, 906*(1), 167548. https://doi.org/10.1016/j.scitotenv.2023.167548

3 Rose, M. T., Patti, A. F., Little, K. R., Brown, A. L., Jackson, W. R., & Cavagnaro, T. R. (2014). A meta-analysis and review of plant-growth response to humic substances. In *Advances in Agronomy*, 37–89. Amsterdam: Elsevier, https://doi.org/10.1016/B978-0-12-800138-7.00002-4

4 Li, Y., Fang, F., Wei, J., Wu, X., Cui, R., Li, G., Zheng, F., & Tan, D. (2019). Humic acid fertilizer improves soil properties and soil microbial diversity of continuous cropping peanut: a three-year experiment. *Scientific Report, 9*, 1–9. https://doi.org/10.1038/s41598-019-48620-46

5 Arif, M., Alagawany, M., Abd El-Hack, M. E., Saeed, M., Arain, M.A., & Elnesr, S.S. (2019). Humic acids as a feed additive in poultry diets: a review. *Iran J Vet Res., 20*(3), 167–172.

6 Savelyeva, A. V., Linkevich, E. V., Yudina, N. V., & Nebogina, N. (2019). Detoxication of oil-contaminated soils by using humic acids. *IOP Conference. Series Materials Science and Engineering, 597*(1), 012020. https://doi.org/10.1088/1757-899X/597/1/012020

7 Tang, W. W., Zeng, G. -M., Gong, J. -L., Liang, J., Xu, P., Zhang, Ch., & Huang, B. -B. (2014). Impact of humic/fulvic acid on the removal of heavy metals from aqueous solutions using nanomaterials: a review. *Sci Total Environ., 15*(468-469), 1014–1027. https://doi.org/10.1016/j.scitotenv.2013.09.044

8 Clapp, C. E., Chen, Y., Hayes, M. H. B., & Cheng, H. H. (2001). Plant growth promoting activity of HS. *Understanding and Managing Organic Matter in Soils, Sediments, and Waters*, 243–255. Madison: IHSS.

9 Kovalevski, D. V., Perminova, I. V., Kudryavtsev, A. V., & Petrosyan, V. S. (1996). A new approach to description of structure of HS and its application to estimating a quantitative structure — property relationship. *The role of humic substance in the ecosystem and in environmental protection: Trans. 8th Meeting of IHSS*, 99. Wroclav (Poland).

10 Riede, U. N., Zeck-Kapp, G., Freudenberg, N., & et al. (1991). Humate induced activation of human granulocytes. *Virchows Arch. B. Cell Pathol. Incl. Mol. Pathol., 60*(1), 27–34.

11 Gorovaya, A. I., Orlov, D. S., & Shcherbenko, O. V. (1995). Guminovye veshchestva: Stroenie, funktsii, mehanizm deistviia, protektor, svoistva, ekologicheskaia rol [Humic substances: Structure, functions, mechanism of action, protector, properties, ecological role]. *Natsionalnaiia akademiia Nauk Ukrainy, Institut problem prirodopolzovaniia i ekologii — National Academy of Sciences of Ukraine, Institute of Environmental Management and Ecology*, 303. Kyiv: Naukova Dumka [in Russian].

12 Imbaeva, D. S., & Kukhar, E. V. (2020). Vliianie biopreparata na osnove gumata kaliia, obogashchennogo mikroelementami, na organizm laboratornykh zhivotnykh [The effect of a biological preparation based on potassium humate enriched with microelements on the organism of laboratory animals]. *Materialy Mezhdunarodnoi nauchno-teoreticheskoi konferentsii «Seifullinskie chteniia — 16: Molodezhnaia nauka novoi formatsii — budushchee Kazakhstana» — Proceedings of the International Scientific and Theoretical Conference "Seifullin Readings — 16: Youth Science of the New Formation — the Future of Kazakhstan", 1*(3), 243–245 [in Russian].

13 Karpukhin, A. I. (1993). Funktsionalnaia rol kompleksnykh soedinenii v genezise pochv i pitanii rastenii [The functional role of complex compounds in soil genesis and plant nutrition]. *Guminovye veshchestva v biosfere — Humic substances in the biosphere*, 117–126. Moscow: Nauka [in Russian].

14 Pirogovskaya, G. V., Bogomaz, I. A., Shagieva, E. I., Kovalenok, M. F., Kuleshova, S. I., Naumova, G. V., & Raitsina, G. I. (1993). Ispolzovanie guminovykh preparatov v poluchenii mineralnykh udobrenii [Use of humic preparations in the production of mineral fertilizers]. *Guminovie veshchestva v biosfere — Humic substances in the biosphere*, 166–174. Moscow: Nauka [in Russian].

15 Abutova, R. J., & Kozhakhmetov, M. K. (2022). Humic preparation and plant activity. *Journal of water and land development, 55*, 104–108. https://doi.org/10.24452/jwld.2022.142311

16 Zhdanova, G. O., Vyatchina, O. F., Bybin, V. A., Stom, D. I., & Fedoseeva, G. M. (2013). Ispolzovanie *Saccharomyces cerevisiae* dlia otsenki biologicheskoi aktivnosti lekarstvennykh preparatov [Use of *Saccharomyces cerevisiae* for assessing the biological activity of medicinal products]. *Sibirskii meditsinskii zhurnal — Siberian Medical Journal, 4*, 104–106 [in Russian].

17 Meyer, B. N., Ferrighi, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica, 45*, 31–34 [in Russian].

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Review

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Anatomical and morphological characteristics of *Ferula varia* growing in Karaganda region (Central Kazakhstan)

The study of plant structure makes it possible to identify characteristic features necessary for species identification and understanding of biological traits. For potentially medicinal plant raw materials, morphological and anatomical studies are an essential component of pharmacognostic analysis. The aim of this study is to investigate the structural features of the above-ground and underground organs of *Ferula varia*, a plant used in folk medicine, at both macro- and microscopic levels. The study was conducted on dried, crushed raw materials using staining techniques and the preparation of temporary slides. The results made it possible to describe the shape and structure of the surface of the stem and leaf plate, as well as the structural features of inflorescences and root fragments. Sparse pubescence on the stem and denser pubescence on the abaxial surface of the leaf blade were observed. At the microscopic level, the structural features and arrangement of tissues in the leaf epidermis, as well as transverse sections of the root, leaf, and stem were characterized. The obtained results made it possible to identify diagnostic features at the macro- and microscopic levels, which allows for species identification of both whole and crushed raw materials. The data obtained may be included in the draft regulatory documents for *Ferula varia* plant raw materials.

Keywords: Ferula varia, above-ground and underground organs, medicinal plant raw materials, macro- and microscopy, diagnostic features.

Introduction

The flora of Kazakhstan has a significant number of species [1], many of which have medicinal properties [2]. Among the considerable diversity of plants, those of the genus *Ferula* L. (family Apiaceae) are of practical interest. Species of this genus are perennial plants that usually grow in arid areas such as steppes and deserts [3], often forming extensive thickets with significant reserves of raw materials [4].

Plants of the *Ferula* genus accumulate various groups of biologically active substances in their above-ground and underground organs, such as coumarins, glycosides, essential oils, sesquiterpene lactones, sulfur-containing metabolites, and gums [2, 5–7]. These substances exhibit a wide range of biological activities, such as anticancer, antimicrobial, anthelmintic, anti-inflammatory, antifungal, antihypertensive, antiprotozoal, antiviral, and others [6–11]. Various parts of plants and their components are widely used in medicine to treat diseases of the cardiovascular system, joints, and oncology, as well as an antispasmodic, antimicrobial, diuretic, and analgesic agent.

There are 46 species of the genus *Feula* growing in Kazakhstan [1], of which *Ferula foetida* (Bunge) Regel, *Ferula iliensis* Krasn. ex Korovin., and *Ferula songarica* Pall. ex Willd. are the most widely used [12–14]. There is fragmentary information on the other species [3], but many species have the potential to be used as a source of medicinal raw materials.

In the Karaganda region, significant areas of thickets have been noted for *Ferula varia* (Schrenk) Trautv., which is typical of the deserts and decertified steppes of Northern Balkhash Region. The roots of this plant are used in folk medicine to treat toothache and fever, as an anthelmintic and wound-healing agent, and a decoction of the seeds stimulates lactation [15].

Pharmacognostic studies, in particular, analysis of the morphological and microscopic indicators of raw materials, are necessary for the introduction of this species into official use.

The aim of this study is to conduct an anatomical and morphological analysis of the above-ground and underground organs of Ferula varia and to identify the diagnostic features of potential medicinal raw materials.

Experimental

Samples of *Ferula varia* raw materials (Fig. 1) for research were collected on April 20–23, 2025, 8 km from the village of Torangylyk (Northern Balkhash Region; coordinates of the collection points: N 46.806017; E 74.794949). Raw materials in the budding phase—early flowering—were harvested in the afternoon, in sunny, dry weather. Above-ground organs were cut at a height of 10 cm from the soil surface, and underground organs were dug up at a depth of up to 50 cm. Species identification was carried out by staff of the Botany Department at the Karaganda National Research University named after academician E.A. Buketov. Plant samples are stored in the herbarium of the Faculty of Biology and Geography (acronym QAR), herbarium sheet number QAR 04328. The air-dried raw materials were soaked in hot water and then fixed in a Feling mixture (90 % ethyl alcohol: distilled water: 40 % glycerin in a 1:1:1 ratio) [16].

Figure 1. Flowering plants *Ferula varia*

When describing the morphological characteristics, attention was paid to the surface structure of individual organs, the presence of pubescence, the color of the organs, the degree of dissection, and the structure at the fracture. Plants parts were examined using a Levenghuk macroscope (China).

Surface preparations were made manually, and sections were made on a Delta Optical manual microtome using Pellucid CW75 microtome blades (China). The resulting micro-preparations were viewed on an EX30 microscope, OD400UHW-P camera (China) with a tablet. The photos with measurements of individual tissues were processed using ImageV software. To obtain higher quality preparations, staining was performed using iron chloride and methylene blue [17].

The micro-preparations were described taking into account the recommendations of R. Crang et al. [18] and publications on the anatomy of plants of the genus *Ferula* L. [19–22].

Results and Discussion

Macroscopy. The stem of *Ferula varia* is rounded in cross section, with small ribs (Fig. 2). The surface is smooth, the color of the upper part of the stem is light green, the lower part is light brown, and the fracture is white or beige. The inflorescence is a complex umbel. The umbels are located at the ends of the fruiting shoots. There are two types of umbels: the central umbels are almost sessile, with 10–15(25) rays. The lateral umbels have 13–18 rays, usually on long pedicels. There may be 1–3 small and underdeveloped umbels.

The flowers are yellow, the calyx is without teeth; the petals are oblong-elliptical, slightly narrowed at the top and curled inward, drooping after flowering. The underside of the leaf is rough, densely pubescent with white, protruding simple trichomes. Longer trichomes are concentrated along the central vein. The vein protrudes above the surface of the leaf blade. The color is green, light green. The upper side of the leaf is rough, marked with rare simple white hairs. The main vein runs through the central part of the blade. The color is green.

The root is ovoid, with a solid neck covered with the remains of dead leaves. The raw material consists of pieces of root of various sizes. The surface of the bark is coarsely wrinkled with remnants of lateral roots. The color is brown, and the fracture is loose and white.

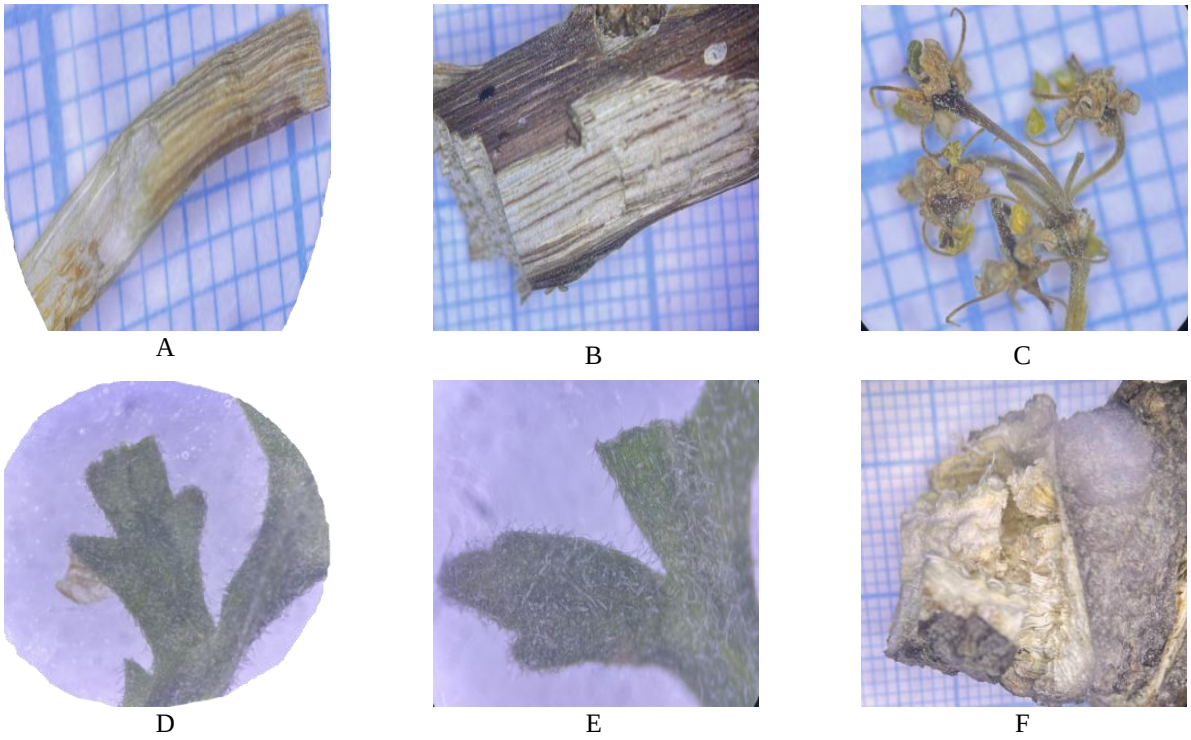

Figure 2. External characteristics of the above-ground and underground organs *of Ferula varia*:
A — stem fragment, B — stem at the break, C — inflorescence fragment,
D — upper side of the leaf, E — lower side of the leaf, F — root fragment

The following diagnostic features can be suggested: stem surface structure, presence of small ribs; shape of second-order umbels; shape of leaf blade, presence of dense pubescence on the underside, color of root and stem at the break.

Microscopy. The stem of *Ferula varia* is rounded in cross section, with small ribs protruding on the surface (Fig. 3). The perimeter of the stem is occupied by a single-layer epidermis consisting of chelicerate cells. Their outer side is thickened and covered with a noticeable layer of cuticle. Rare simple trichomes are found. Under the epidermis lies 2-3 layers of chlorenchyma, which are interrupted under the ribs by small strands of mechanical collenchyma tissue. The bundles are numerous, collateral, closed (phloem and xylem, no cambium). The peripheral bundles are elliptical in shape, with larger and smaller bundles alternating. Medullary bundles, usually round or ovoid in shape, lie in the central parenchyma. The peripheral bundles are covered with small “caps” of sclerenchyma. Schizogenous cavities, oval or rounded in shape, are located around the entire perimeter of the stem, above the bundles. The central part is filled with loose and thin-walled cells of the heart parenchyma.

A similar structure is noted for the stems of *Ferula foetida*, with differences in the shape of the cavities. In *Ferula foetida* [22], the cavities are oval in shape and almost identical in size, while in *Ferula varia*, they are rounded and vary in diameter.

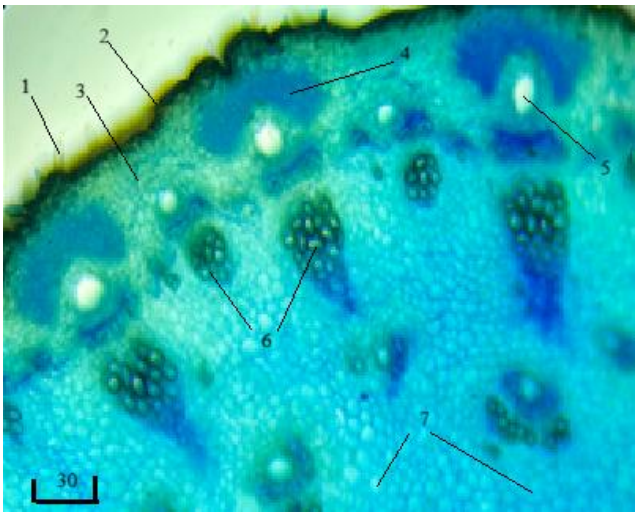

Figure 3. Cross section of the stem of *Ferula varia*, fragment. Staining: methylene blue:
1 — trichome, 2 — epidermis, 3 — cork parenchyma, 4 — chlorenchyma, 5 — vessels,
6 — vascular bundles, 7 — pith parenchyma cells; dimensions in μm

The epidermis of the upper and lower sides of the *Ferula varia* leaf is identical in structure, consisting of elongated cells with straight or slanted walls. Trichomes are rare on the upper side and more abundant on the lower side of the leaf. The stomata are small, few in number, diacytic, and located on both sides—amphistomatic type.

In cross section, the leaf is flat, dorsal-ventral type, with undifferentiated mesophyll on columnar and spongy tissue (Fig. 4). On both sides, the leaf is surrounded by a single-layer epidermis, its cells are oval, covered with a layer of cuticle. Simple unicellular trichomes are clearly visible, especially in the area of the conducting bundles. The inner part is filled with loose mesophyll cells. The central and lateral conducting bundles are collateral, closed type, weakly sclerenchymatized. Around the bundles, there are rounded cavities of schizogenous origin; the shape is rounded.

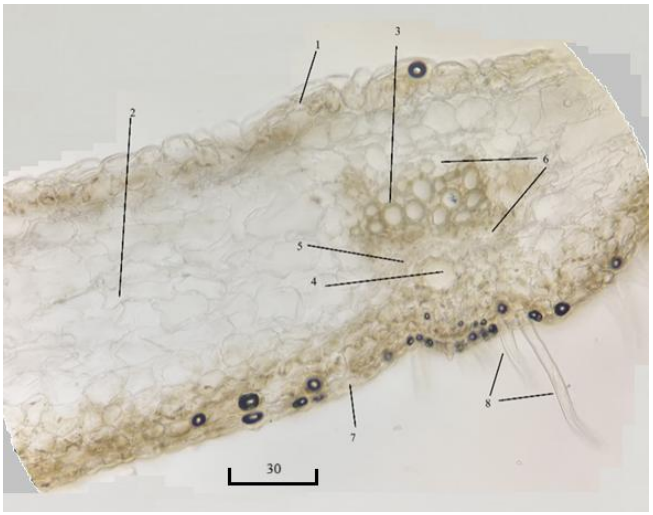

Figure 4. Cross section of *Ferula varia* leaf, fragment in the area of the central vein. Staining iron chloride:
1 — upper epidermis, 2 — mesophyll, 3 — xylem, 4 — cavity, 5 — phloem,
6 — conducting bundle, 7 — lower epidermis, 8 — simple trichomes; dimensions in μm

A poorly differentiated mesophyll is also characteristic of other *Ferula* species, as is a small number of vessels.

The root in cross section is covered with a multilayered cork, its cells are dark brown, almost rectangular (Fig. 5). Under the bark lies a multilayered cortical parenchyma. The endoderm is single-layered and consists of oval-shaped cells. The central cylinder of the root consists of a complex radial-type bundle in which

xylem and phloem rays alternate. In some places, there are areas of pericycle. In the inner part of the root, there are numerous cavities of schizogenous origin, usually round in shape.

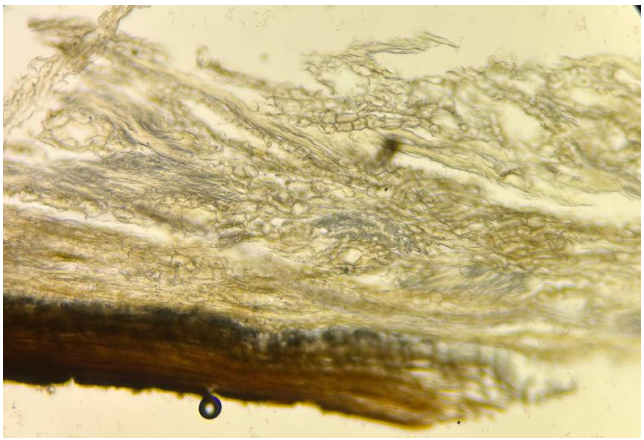

Figure 5. Cross section of the root of *Ferula varia*, fragment

The structure of the root of *Ferula varia* differs significantly from that of *Ferula foetida* [22], as it forms a denser structure that does not break down into separate fragments. The cavities in the underground organs have a similar structure to other species of ferula [20–22].

Analysis of microscopic indicators has made it possible to identify diagnostic features that can help in the identification of crushed raw materials:

-for the stem: the shape of the stem in cross section, the shape of the conducting bundles, areas of collenchyma, the rounded shape and different diameters of the vessels;

-for the root: the presence of rounded cavities of schizogenous origin;

-for the leaf: undifferentiated mesophyll, the shape of the conducting bundles, the presence of rounded vessels, dense pubescence on the underside of the leaf; the presence of simple trichomes.

Conclusion

Thus, based on the results of the studies, the morphological and anatomical features of the above-ground and underground organs of *Ferula varia* were determined. Characteristic features of the structure at the macroscopic level were noted for the shape of the leaves, the structure of the stem surface, and the shape of the secondary umbels.

The above-ground organs are characterized by the primary anatomical structure of the stem with the presence of peripheral collateral and medullary conducting bundles. The leaves are classified as dorsoventral with undifferentiated mesophyll and denser pubescence on the underside of the leaf blade. The root is characterized by a secondary structure, a thick layer of cork, and a radial arrangement of conducting elements. The presence of round-shaped schizogenous cavities was determined for all organs studied.

The obtained results made it possible to identify diagnostic features at the macro- and microscopic levels, which allows for species identification of both whole and crushed raw materials.

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Conflict of interest

The authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions from all authors. All authors have given approval to the final version of the manuscript: **Sabiyeva A.** — conceptualization, data analysis, investigation; **Smagulov N.K.** — plant material collecting, writing draft, data curation; **Atazhanova G.A.** — methodology, morphological investigation; **Turdiyeva Zh.A.** — anatomical investigation, data analysis.

References

1 Флора Казахстана. ТТ. 1–9. — Алма-Ата: Наука, 1656–1966.

2 Грудзинская Л.М. Аннотированный список лекарственных растений Казахстана: Справочное издание / Л.М. Грудзинская, Н.Г. Гемеджиева, Н.В. Нелина, Ж.Ж. Каржаубекова. — Алматы, 2014. — 200 с.

3 Сафина Л.К. Ферулы Казахстана / Л.К. Сафина, М.Г. Пименов. — Алма-Ата: Наука, 1984. — 110 с.

4 Каирова М.Ж. Изучение популяции *Ferula foetida* (Bunge) Regel, произрастающей в горах Хан-Тау / М.Ж. Каирова, А.А. Шорманова, Г.Т. Ситпаева // Вестник КазНУ, серия биол. — 2023. — № 4(97). — С. 51–60. https://doi.org/10.26577/eb.2023.v97.i4.05

5 Mohammadhosseini M. The genus *Ferula*: ethnobotany, phytochemistry and bioactivities — a review / M. Mohammadhosseini, A. Venditti, S.D. Sarker, L. Nahar, A. Akbarzade // Industrial Crops and Practice. — 2019. — Vol. 129. — P. 350–394. https://doi.org/10.1016/j.indcrop.2018.12.012

6 Wang J. Sesquiterpenes and sesquiterpene derivates from *Ferula*: their chemical structures, biosynthetic pathways, and biological properties / J. Wang, Q. Zheng, H. Wang, L. Shi, G. Wang, Y. Zhao, C. Fan, J. Si // Antioxidants. — 2023. — Vol. 13(1). https://doi.org/10.3390/antiox13010007

7 Sonigra P. Methabolic profile, bioactivities, and variations in the chemical constituents of essential oils of the Ferula genus (Apiaceae) / P. Sonigra, M. Meena // Front in Pharmacol. — 2021. — Vol. 11. https://doi.org/10.3389/fphar.2020.608649

8 Iranshahi M. Cytotoxic activity of the genus *Ferula* (Apiaceae) and its bioactive constituents / M. Iranshahi, R. Rezaee, M.N. Najafi, A. Haghbin, J. Kasaian // Avicenna J Phytomed. — 2018. — Vol. 8(4). — P. 296–312.

9 Amalraj A. Biological activities and medicinal properties of Asafoetida: a review / A. Amalraj, S. Gopi // J Tradit Complement Med. — 2016. — Vol. 7(3). — P. 347–359. https://doi.org/10.1016/j.jtcme.2016.11.004

10 Boghrati Z. *Ferula* species: a rich source of antimicrobial compounds / Z. Boghrati, M. Iranshahi // Journal of Herbal Medicine. — 2019. — Vol. 16. https://doi.org/10.1016/j.hermed.2018.10.009

11 Ghasemi Z. Anti-inflammatory, anti-oxidant, and immunomodulatory activities of the genus *Ferula* and their constituents: a review / Ghasemi Z., R. Rezaee, M.R. Aslani, M.H. Boskabady // Iran J Basic Med Sci. — 2021. — Vol. 24(12). — P. 1613–1623. https://doi.org/10.22038/IJBMS.2021.59473.13204

12 Байтулин И.О. О некоторых хозяйственно-ценных видах р. Ferula Казахстана / И.О. Байтулин, А.М. Нурушева // Известия НАН РК, серия биол. и медиц. — 2008. — № 6. — С. 3–6.

13 Турдиева Ж.А. Антимикробная активность эфирного масла *Ferula soongarica*, произрастающей в Центральном Казахстане / Ж.А. Турдиева, Г.А. Атажанова, М.Ю. Ишмуратова, С.Б. Ахметова // Вестник «Южно-Казахстанской медицинской академии». — 2022. — № 4 (98). — С. 68-69.

14 Саидова Н.Г. Лечебное растение ферула вонючая / Н.Г. Саидова, Г.Х. Кодирова, И.Д. Кароматов // Биология и интегративная медицина. — 2017. — № 9. — С. 58–77.

15 Котенко Л.Д. Стандартизация травы ферулы изменчивой / Л.Д. Котенко, М.А. Маматханова, Р.М. Халилов, А.У. Маматханов, Г.Б. Сотимов // Химия растительного сырья. — 2009. — № 4. — С. 151–154.

16 Барыкина Р.П. Справочник по ботанической микротехнике. Основы и методы / Р.П. Барыкина, Т.Д. Веселова, А.Г. Девятов, Х.Х. Джалилова, Г.М. Ильина, Н.В. Чубатова. — Москва: Изд-во МГУ, 2004. — 312 р.

17 Rudall P.J. Anatomy of flowering plants / P.J. Rudall. — Cambridge: Cambridge University Press, 2007. — 159 p.

18 Crang R. Plant anatomy: a concept-based approach to the structure of seed plants / R. Crang, Sh. Lyons-Sobaski, R. Wise. — Springer, 2018. — 507 p.

19 Liu M.-M. Microscopic anatomy and ultrastucture of the resin ducts of *Ferula ferulaeoides* (Steud.) Korov. in Xinjiang / M.-M. Liu, S.-W. Zhang, Q.-G. Liu, M.-M. Guo // Microscopic Research and Technique. — 2020. — Vol. 83(12). — P. 1566–1573. https://doi.org/10.1002/jemt.23552

20 Sagyndykova M.S. Study of the morphological and anatomical structure of *Ferula foetida* seeds from different Mangyshlak populations / M.S. Sagyndykova, A. A Imanbaeva, M. Yu Ishmuratova // Plant Cell Biotechnology and Molecular Biology. — 2020. — Vol. 21 (15-16). — P. 150–156.

21 Khamraeva D.T. Comparative anatomical study of underground and aboveground organs in *Ferula tadshikorum* Pimenov under natural and introduced environment / D.T. Khamraeva, D.N. Tukhtaeva, P.K. Khojimatov, R.W. Bussmann // Acta Biologica Sibirica. — 2024. — Issue 10. — P. 9–29. https://doi.org/10.5281/zenodo.10475286

22 Imanbayeva A.A. Anatomical organization of above- and underground organs of *Ferula foetida* (Bunge) Regel in Mangistau natural population / A.A. Imanbayeva, K.N. Sarsenbayev, M.S. Sagyndykova // Contemporary Problems of Ecology. — 2015. — Vol. 8(6). — P. 743–753. https://doi.org/10.1134/S1995425515060086

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Қарағанды облысында (Орталық Қазақстан) өсетін *Ferula varia* өсімдігінің анатомиялық және морфологиялық ерекшеліктері

Өсімдіктердің құрылысын зерттеу олардың түрлік тиесілігін анықтау және биологиялық ерекшеліктерін түсіну үшін қажет болатын өзіне тән белгілерін айқындауға мүмкіндік береді. Потенциалды дәрілік өсімдік шикізаты үшін морфологиялық-анатомиялық зерттеулер жүргізу фармакогностикалық талдаудың міндетті компоненті. Зерттеудің мақсаты халық медицинасында қолданылатын *Ferula varia* өсімдігінің жерүсті және жерасты мүшелерінің құрылымдық ерекшеліктерін макро- және микроскопиялық деңгейде зерттеу. Зерттеулер кептірілген және ұнтақталған шикізатқа бояу әдістерін қолдану және уақытша микропрепараттар дайындау арқылы жүргізілді. Нәтижелер сабақ пен жапырақ тақтасының пішіні мен беткі құрылымын, гүл шоғырларының құрылымдық ерекшеліктерін, сондай-ақ тамыр бөліктерінің анатомиялық сипаттамаларын анықтауға мүмкіндік берді. Сабақтың сирек түкті екендігі, ал жапырақ тақтасының төменгі жағында түктенудің анағұрлым тығыз екендігі анықталды. Микроскопиялық деңгейде жапырақ эпидермисі тіндерінің құрылысы мен орналасу ерекшеліктері, сондай-ақ тамырдың, жапырақтың және сабақтың көлденең кесінділерінің анатомиялық сипаттамалары анықталды. Алынған нәтижелер макро- және микроскопиялық деңгейде диагностикалық белгілерді айқындауға мүмкіндік берді, бұл бүтін және ұнтақталған шикізаттың түрлік сәйкестендірілуін жүргізуге жағдай жасайды. Жиналған деректер *Ferula varia* өсімдік шикізатына арналған нормативтік құжаттар жобасын әзірлеуде пайдаланылуы мүмкін.

Кілт сөздер: Ferula varia, жерүсті және жерасты мүшелер, дәрілік өсімдік шикізаты, макро- және микроскопия, диагностикалық белгілер

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Анатомические и морфологические особенности *Ferula varia,* произрастающей в Карагандинской области (Центральный Казахстан)

Изучение строения растений позволяет выделять характерные признаки, что необходимо для идентификации видовой принадлежности и понимания биологических особенностей. Для потенциального лекарственного растительного сырья проведение морфолого-анатомических исследований является обязательным компонентом фармакогностического анализа. Цель настоящего исследования — провести изучение особенностей строения надземных и подземных органов *Ferula varia*, применяемой в народной медицине, на макро- и микроскопическом уровне. Исследования проводили для высушенного измельченного сырья с применением окрашивания и приготовления временных препаратов. Результаты позволили описать форму и структуру поверхности стебля и листовой пластины, особенности строения соцветий и фрагментов корня. Определено наличие редкого опушения стебля, более густого — на нижней стороне листовой пластины. На микроскопическом уровне установлены особенности строения и расположения тканей эпидермиса листа, поперечных срезов корня, листа и стебля. Полученные результаты позволили выделить диагностические признаки на макро- и микроскопическом уровне, что позволяет проводить видовую идентификацию как цельного, так и измельченного сырья. Полученные данные могут войти в проект нормативных документов на растительное сырье *Ferula varia*.

Ключевые слова: *Ferula varia*, надземные и подземные органы, лекарственное растительное сырье, макро- и микроскопия, диагностические признаки

References

1 (1956–1966). *Flora Kazakhstana* [Flora of Kazakhstan]. Alma-Ata: Nauka [in Russian].

2 Grudzinskaya, L.M., Gemedzhieva, N.G., Nelina, N.V., & Karzhaubekova Zh.Zh. (2014). *Annotirovannyi spisok lekarstvennykh rastenii Kazakhstana: spravochnoe izdanie* [Annotated list of medicinal plants of Kazakhstan: Reference edition]. Almaty [in Russian].

3 Safina, L.K., & Pimenov, M.G. (1984). *Feruly Kazakhstana* [Ferulas of Kazakhstan]. Alma-Ata: Nauka [in Russian].

4 Kairova, M.Zh., Shormonova, A.A., & Sitpaeva, G.T. (2023). Izuchenie populiatsii *Ferula foetida* (Bunge) Regel, proizrastaiushchei v gorakh Han-Tau [Study of the population *of Ferula foetida* (Bunge) Regel growing in the Khan-Tau mountains]. *Vestnik Kazakhskogo natsionalnogo universiteta. Seriia biologiia — Bulletin of Kazakh National University. Biology series, 4*(97), 51–60. https://doi.org/10.26577/eb.2023.v97.i4.05 [in Russian].

5 Mohammadhosseini, M., Venditti, A., Sarker, S.D., Nahar, L., & Akbarzade, A. (2019). The genus *Ferula*: ethnobotany, phytochemistry and bioactivities — a review. *Industrial Crops and Practice, 129,* 350–394. *https://doi.org/10.1016/j.indcrop.2018.12.012*

6 Wang, J., Zheng, Q., Wang, H., Shi, L., Wang, G., Zhao, Y., Fan, C., & Si, J. (2023). Sesquiterpenes and sesquiterpene derivates from *Ferula*: their chemical structures, biosynthetic pathways, and biological properties. *Antioxidants, 13*(1). https://doi.org/10.3390/antiox13010007

7 Sonigra, P., & Meena, M. (2021). Methabolic profile, bioactivities, and variations in the chemical constituents of essential oils of the *Ferula* genus (Apiaceae). *Front in Pharmacol., 11*. https://doi.org/10.3389/fphar.2020.608649

8 Iranshahi, M., Rezaee, R., Najafi, M. N., Haghbin, A., & Kasaian, J. (2018). Cytotoxic activity of the genus *Ferula* (Apiaceae) and its bioactive constituents. *Avicenna J Phytomed., 8*(4), 296–312.

9 Amalraj, A., & Gopi, S. (2016). Biological activities and medicinal properties of Asafoetida: a review. *J Tradit Complement Med., 7*(3), 347–359. https://doi.org/10.1016/j.jtcme.2016.11.004

10 Boghrati, Z., & Iranshahi, M. (2019). *Ferula* species: a rich source of antimicrobial compounds. *Journal of Herbal Medicine, 16*. https://doi.org/10.1016/j.hermed.2018.10.009

11 Ghasemi, Z., Rezaee, R., Aslani, M. R., & Boskabady, M. H. (2021). Anti-inflammatory, anti-oxidant, and immunomodulatory activities of the genus *Ferula* and their constituents: a review. *Iran J Basic Med Sci., 24*(12), 1613–1623. https://doi.org/10.22038/IJBMS.2021.59473.13204

12 Baitulin, I.O., & Nurusheva, A.M. (2008). O nekotorykh khoziaistvenno-tsennykh vidakh r. *Ferula* Kazakhstana [On some economically valuable species of Ferula in Kazakhstan]. *Izvestiia Natsionalnoi Akademii Nauk Respubliki Kazakhstan, Seriia biologiia i meditsina — Proceedings of the National Academy of Sciences of Kazakhstan, Biology and Medicine Series,* 6, 3–6 [in Russian].

13 Turdieva, Zh.A., Atazhanova, G.A., Ishmuratova, M.Yu., & Akhmetova, S.B. (2022). Antimikrobnaia aktivnost efirnogo masla *Ferula soongarica*, proizrastaiushchei v Tsentralnom Kazakhstane [Antimicrobial activity of essential oil *of Ferula soongarica* growing in Central Kazakhstan]. *Vestnik «Yuzhno-Kazakhstanskoi meditsinskoi akademii» — Bulletin of the South Kazakhstan Medical Academy, 4*(98), 68-69 [in Russian].

14 Saidova, N.G., Kodiova, G.Kh., & Karomatov, I.D. (2017). Lechebnoe rastenie ferula voniuchaia [The medicinal plant *Ferula foetida*]. *Biologiia i integrativnaia meditsina — Biology and Integrative Medicine,* 9, 58–77 [in Russian].

15 Kotenko, L.D., Mamatkhanova, M.A., Khalilov, R.M., Mamatkhanov, A.U., & Sotimov, G.B. (2009). Standartizatsiia travy feruly izmenchivoi [Standardization of the herb *Ferula varia*]. *Khimiia rastitelnogo Syria — Chemistry of Plant Raw Materials,* 4, 151–154 [in Russian].

16 Barykina, R.P., Veselova, T.D., Devyatov, A.G., Dzhalilova, H.H., Ilyina, G.M., & Chubatova, N.V. (2004). *Spravochnik po botanicheskoi mikrotekhnike. Osbovy i metodi* [Handbook of Botanical Microtechnology. Fundamentals and Methods]. Moscow: Izdatelstvo Moskovskogo gosudarstvennogo universiteta [in Russian].

17 Rudall, P.J. (2007). *Anatomy of flowering plants*. Cambridge: Cambridge University Press.

18 Crang, R., Lyons-Sobaski, Sh., & Wise, R. (2018). *Plant anatomy: a concept-based approach to the structure of seed plants*. Springer.

19 Liu, M. -M., Zhang, S.-W., Liu, Q.-G., & Guo, M.-M. (2020). Microscopic anatomy and ultrastructure of the resin ducts of *Ferula ferulaeoides* (Steud.) Korov. in Xinjiang. *Microscopic Research and Technique, 83*(12), 1566–1573. https://doi.org/10.1002/jemt.23552

20 Sagyndykova, M. S., Imanbaeva, A. A., & Ishmuratova, M. Yu. (2020). Study of the morphological and anatomical structure of *Ferula foetida* seeds from different Mangyshlak populations. *Plant Cell Biotechnology and Molecular Biology, 21* (15-16), 150–156.

21 Khamraeva, D.T., Tukhtaeva, D.N., Khojimatov, P.K., & Bussmann, R.W. (2024). Comparative anatomical study of underground and aboveground organs in *Ferula tadshikorum* Pimenov under natural and introduced environment. *Acta Biologica Sibirica,* 10, 9–29. https://doi.org/10.5281/zenodo.10475286

22 Imanbayeva, A.A., Sarsenbayev, K.N., & Sagyndykova, M.S. (2015). Anatomical organization of above- and underground organs of *Ferula foetida* (Bunge) Regel in Mangistau natural population. *Contemporary Problems of Ecology, 8*(6), 743–753. https://doi.org/10.1134/S1995425515060086

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