

Research Article

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Bacterial and Fungal Contamination during Garlic (*Allium sativum* L.) Initiation in In Vitro Culture

The present study investigated bacterial and fungal contamination occurring during the establishment of garlic (*Allium sativum* L.) in vitro cultures. The aim of the study was to assess the level of contamination in explants and evaluate the effectiveness of standard surface sterilization protocols. The scientific novelty of the study lies in the first comprehensive assessment in Kazakhstan of bacterial and fungal contamination occurring during the establishment of garlic in vitro cultures. The experiments demonstrated that conventional sterilization methods, including ethanol and disinfectant treatments, did not ensure complete decontamination of the plant material. Consequently, microbial contamination was observed at the early stages of cultivation, resulting in partial or complete necrosis of explants and reduced efficiency of micropropagation. The degree of contamination varied among garlic cultivars, suggesting an influence of genotype and the physiological condition of the donor plants. Microscopic analysis indicated the predominance of fungal morphotypes consistent with genera such as *Fusarium*, *Penicillium*, and *Aspergillus*, as well as the presence of diverse bacterial microflora. However, accurate taxonomic identification of the contaminants requires further molecular genetic analysis. The results demonstrate that microbial contamination remains a critical limiting factor for the successful establishment of garlic in vitro cultures. Therefore, improved pre-treatment of plant material, optimization of sterilization protocols, and refinement of culture conditions are required. The practical significance of the study lies in the development of more effective approaches to reducing contamination, thereby increasing the success rate of micropropagation and facilitating the establishment of healthy in vitro collections.

Keywords: garlic, *Allium sativum* L., in vitro, microbial contamination, explants, *Fusarium*, *Penicillium*.

Introduction

Garlic (*Allium sativum* L.) is a vegetatively propagated crop, which facilitates the accumulation and persistence of infectious agents in planting material. A significant proportion of phytopathogens may remain latent during the growing season; however, they become activated during storage, leading to substantial post-harvest losses [1].

According to numerous studies, the most common pathogens affecting garlic include fungi of the genera *Fusarium*, *Penicillium*, *Botrytis*, and *Sclerotium*, as well as bacteria belonging to the genera *Pectobacterium*, *Pantoea*, and *Pseudomonas* [2, 3]. The species composition of pathogens and the degree of infection vary depending on the soil and climatic conditions of the cultivation region.

A particular concern is the presence of latent internal infections that persist within bulb tissues. Even when standard surface sterilization protocols are applied during the introduction of plants into in vitro culture, microorganisms localized in internal tissues may become activated and cause contamination of explants. This results in necrosis, tissue decay, and reduced efficiency of micropropagation [4, 5].

Fungal diseases, including fusariosis, penicilliosis, neck rot, and white rot, are among the main factors reducing the quality of planting material during storage. The mycelium of pathogens can spread through the vascular system of plants, block conductive tissues, and produce toxic metabolites, thereby intensifying degenerative processes. Bacterial infections, in turn, are primarily manifested as soft rot affecting cloves and the basal plate of bulbs [6].

Preventive measures, including quarantine, removal of infected plants, and control of natural vectors, remain the primary strategies for limiting pathogen spread. Unlike other agricultural crops, where modern

approaches to developing genetic resistance—such as transformation, genome editing, or cross-protection—are applied, their use in garlic is restricted due to the lack of efficient regeneration systems.

Therefore, maintaining the phytosanitary purity of planting material relies primarily on pathogen elimination and the cultivation of healthy plants under controlled conditions. Since garlic is vegetatively propagated and does not produce viable seeds, obtaining pathogen-free material is achievable through in vitro methods, such as meristem culture, thermotherapy, chemotherapy, and cryotherapy [7, 8].

Bacterial contamination is typically manifested by turbid growth of the medium, unpleasant odors, and meristem necrosis [9]. Fungal contamination appears as mycelial growth on the surface of the substrate or plant tissues, which also leads to the death of explants. The primary sources of contamination are endophytic microorganisms residing within garlic cloves, as well as spores of microorganisms present on the surface [10–12].

Several approaches are employed to minimize the risk of contamination, including meticulous sterilization of the starting material, the use of antimicrobial agents, careful control of culture conditions, and the selection of cultivars with high resistance to infections. Despite these measures, contamination remains a significant challenge, affecting the efficiency of garlic micropropagation and the quality of the resulting in vitro collection. Therefore, addressing bacterial and fungal contamination is a key aspect of successful garlic introduction into in vitro culture and requires an integrated approach using modern sterilization techniques and microorganism control methods [13, 14].

Effective contamination management is essential for establishing a healthy in vitro garlic collection, particularly when working with infection-sensitive cultivars, and for the subsequent application of micropropagation in breeding and biotechnological programs [15, 16]. Consequently, a comprehensive investigation of bacterial and fungal contamination in garlic planting material following low-temperature storage, prior to its introduction into in vitro culture, is highly relevant.

Experimental

The study was conducted in the biotechnology laboratory of the “Potato Breeding, Seed Production, and Biotechnology” Department at the Kazakh Research Institute of Horticulture, Branch “Kainar”, during 2024–2025.

Three garlic (*Allium sativum* L.) cultivars “Arman”, “Zailiyskiy”, and “Dunganskiy” were used as research objects. Visually healthy cloves were selected for in vitro culture, and the basal plate portion was used as explants.

The plant material was stored in a dry environment at approximately 10 °C for 7–8 months prior to the experiment. Culture initiation was carried out under aseptic conditions in a laminar flow cabinet.

All manipulations were performed under aseptic conditions using a laminar airflow cabinet (ESCO, Singapore). Sterilization was carried out using an autoclave (Tuttnauer, Germany) at a temperature of 121 °C, a pressure of 1.1 atm, and an exposure time of 20 min to ensure complete sterility of culture media and instruments. Microscopic analysis was conducted using a light microscope (Olympus, Japan).

Sterilization of the plant material was performed in two stages. The first stage involved the removal of external contaminants: washing the plant material in running water for 30–60 min, soaking the cloves in a 10 % solution of household soap for 40–60 min, followed by rinsing in running water and immersion in a 0.1 % potassium permanganate solution for 15–20 min.

The second stage was performed under laminar flow conditions and included surface sterilization of explants with 70 % ethanol for 30 s, followed by immersion in 1 % sodium hypochlorite solution for 20 min, and subsequent rinsing three times with sterile distilled water. This sterilization protocol was applied to reduce external microbial contamination and ensure aseptic conditions for in vitro culture initiation [17, 18].

The experiment was conducted in three biological replicates, each comprising 13–15 explants. Explants were cultured on Murashige and Skoog (MS) medium supplemented with 3 % sucrose and 0.7 % agar, adjusted to pH 5.8. Cultures were maintained at 24 ± 2 °C under a 16/8 h photoperiod with a light intensity of $40\text{--}50 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Contamination levels were assessed on days 3, 7, and 14 of cultivation. Statistical analysis was performed using Microsoft Excel. Data are presented as mean values calculated from replicates. Microorganism identification was carried out based on morphological characteristics using diagnostic keys; however, the results are considered preliminary and require confirmation by molecular genetics methods.

Microorganism identification was additionally performed using light microscopy and requires further validation by molecular techniques, including PCR analysis using 16S rRNA markers for bacteria and ITS regions for fungi.

The percentage of contamination was calculated as the ratio of contaminated explants to the total number of cultured explants. Results are presented as mean $\pm$ standard deviation. Differences among cultivars were analyzed using analysis of variance (ANOVA), followed by Tukey's post hoc test to determine statistically significant differences ($p < 0.05$). Each experiment was conducted in three biological replicates.

Results and Discussion

The introduction of cloves from the “Arman”, “Zailiyskiy”, and “Dunganskiy” garlic cultivars into in vitro culture revealed that explants contamination by various phytopathogens was observed within the first week. Bacterial growth was detected by day 3, while fungal pathogen development became evident after 6-7 days (Fig. 1).

Table 1

Contamination Levels of Explants of Different Garlic Cultivars

№	Garlic Cultivar	Total Number of Explants	Number of Infected Explants	<i>Fusarium</i> , %	<i>Penicillium</i> , %	<i>Aspergillus</i> , %	<i>Pseudomonas</i> , %	Total Infected, %
1	Arman	45	35	64.5	14.2	9.1	12.1	77.8
2	Zailiyskiy	44	29	25.4	28.7	13.0	31.0	65.9
3	Dunganskiy	41	28	26.3	32.1	9.2	33.2	68.5

The results presented in the table characterize the structure of microbial contamination of garlic explants by cultivar and allow for the assessment of the contribution of individual pathogen groups to the overall level of infection. The analysis included fungi of the genera *Fusarium*, *Penicillium*, and *Aspergillus*, as well as bacteria of the genus *Pseudomonas*. The indicators reflect the relative prevalence of the identified microorganisms and their share in the total infection of the studied samples.

The highest proportion of infected explants was observed in the “Arman” cultivar, which is associated with the particular internal tissue structure and the accumulation of pathogens during storage. Overall, differences in cultivar sensitivity to fungal and bacterial infections were noted.

Fungi of the genera *Fusarium*, *Penicillium*, and *Aspergillus*, as well as bacteria of the genus *Pseudomonas*, were detected in the garlic explants. In the “Arman” cultivar, *Fusarium* (64.5 %) and *Pseudomonas* (12.1 %) predominated. In the “Zailiyskiy” cultivar, *Penicillium* (28.7 %) and *Fusarium* (25.4 %) were the main fungal pathogens, while *Pseudomonas* bacteria accounted for 31.0 % of contamination. In the “Dunganskiy” cultivar, the most frequently observed microorganisms were *Penicillium* (32.1 %), *Fusarium* (26.3 %), and *Pseudomonas* (33.2 %).

The observed differences in pathogenic microflora composition are associated with the biological traits of the cultivars. Cultivars with less developed aerial bulbs and weak scape formation tend to accumulate higher levels of fungal and bacterial infections in their internal tissues (Fig. 2). Despite rigorous surface sterilization, internal infections persisted and became apparent in the explants during in vitro culture (Tab. 2).

Table 2

Average Percentage of Bacterial and Fungal Contamination of Garlic Explants

№	Garlic Cultivar	Bacterial Contamination (<i>Pseudomonas</i>), % $\pm$ SD	Fungal Contamination (<i>Fusarium</i> + <i>Penicillium</i> + <i>Aspergillus</i>), % $\pm$ SD
1	Arman	12.1 $\pm$ 1.0	87.8 $\pm$ 2.5
2	Zailiyskiy	31.0 $\pm$ 2.0*	65.9 $\pm$ 2.0*
3	Dunganskiy	33.2 $\pm$ 1.8*	68.5 $\pm$ 2.1*

*Differences were considered statistically significant at $p < 0.05$.

The obtained results indicate the persistence of latent microbial infections in garlic planting material, which become evident at the early stages of in vitro culture (Fig. 1). Contamination levels varied among cultivars, which should be taken into account when organizing micropropagation and the sanitation of planting material.



Figure 1. In vitro culture of garlic showing *Penicillium* contamination. Scale bar = 0.1 cm

In Figure 2A, colonies of *Penicillium* can be observed developing in the lower part of the plant material, indicating partial contamination. This highlights the necessity of thorough sterilization and strict control of explant cleanliness during in vitro culture.

In Figure 2B, branched conidia and the characteristic mycelium of *Penicillium* are visible, confirming contamination at the cellular level. This image emphasizes the need for strict control of medium sterility and careful material sterilization prior to culture initiation.

In Figure 3, sickle-shaped multicellular macroconidia of *Fusarium* isolated from infected garlic (*Allium sativum* L.) tissues are shown. The conidia exhibit an elongated, curved shape with 3–5 transverse septa, tapered ends, and a hyaline structure. The observed morphological characteristics are typical of *Fusarium* species and confirm the fungal nature of the infection.

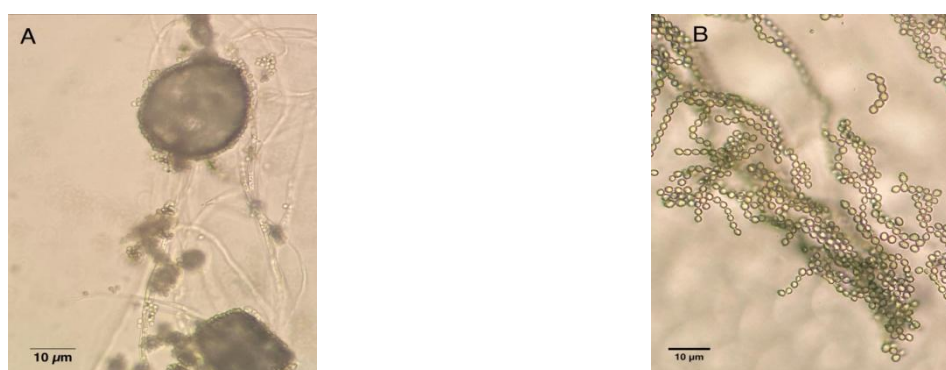


Figure 2. Microscopic image of *Penicillium* colonies on a garlic (*Allium sativum* L.) explant in in vitro culture. (A) Fungal structures observed on explant surface. (B) Abundant fungal hyphae and spores indicating contamination. Magnification $\times 600$. Scale bar = 10 μm

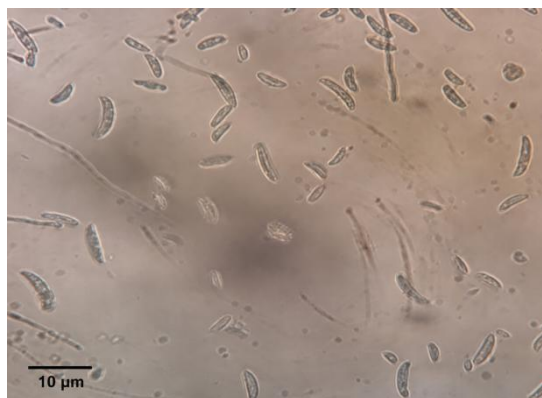


Figure 3. Macroconidia of *Fusarium* isolated from infected garlic (*Allium sativum* L.) tissues. Magnification $\times 600$. Scale bar = 10 μm

These results are consistent with previous studies indicating the widespread occurrence of latent infections in garlic planting material. The higher contamination levels observed in certain cultivars may be associated with anatomical features of the tissues, as well as prolonged low-temperature storage, which promotes the development of hidden microflora. At the same time, the incomplete decontamination following surface sterilization suggests possible localization of microorganisms within internal tissues; however, additional studies, including isolation of pure cultures, are required to confirm the endophytic nature of the infection.

The results obtained indicate that contamination of garlic explants becomes apparent at the early stages of in vitro culture, which is consistent with previous studies reporting the widespread occurrence of latent infections in vegetatively propagated crops. The early onset of bacterial contamination (by day 3) followed by the subsequent development of fungal microflora reflects the different growth dynamics of microorganisms under in vitro conditions.

The observed predominance of fungi of the genera *Fusarium*, *Penicillium*, and *Aspergillus*, as well as bacteria of the genus *Pseudomonas*, aligns with literature data on the most common garlic phytopathogens associated with storage and subsequent cultivation. This confirms that even visually healthy planting material may harbor latent infections.

Differences in contamination levels and structure among cultivars suggest a possible influence of plant genotypic characteristics, including tissue anatomy, density of protective layers, and their permeability to microorganisms. Additionally, prolonged storage at low temperatures may have contributed to the persistence and partial activation of latent microflora.

It was established that the application of the standard surface sterilization protocol does not ensure complete decontamination of explants. This suggests a likely localization of microorganisms within internal tissues (endophytic infection), which are inaccessible to sterilizing agents. Similar conclusions have been reported in studies on the cultivation of garlic and other vegetatively propagated crops.

It should be noted that microorganism identification in this study was based on morphological characteristics, which limits the accuracy of taxonomic assignment, particularly for bacterial microflora. Therefore, further research should focus on molecular-genetic identification using PCR and sequencing methods.

A limitation of the present study is the relatively small sample size and the descriptive nature of the statistical analysis, which does not allow a full assessment of the significance of differences between cultivars. Nevertheless, the obtained data provide a general overview of contamination structure and can serve as a basis for further investigations.

The practical significance of this work lies in the necessity of considering the identified contamination features when developing protocols for introducing garlic into in vitro culture. Promising directions include optimization of sterilization schemes, including the use of combined methods (thermotherapy, antibiotics, fungicides), as well as the preliminary selection of planting material with a low level of infection.

Thus, the results of this study confirm the complex and multifactorial nature of contamination during in vitro garlic cultivation and highlight the need for a comprehensive approach to address this issue.

Conclusion

The conducted study demonstrated that garlic planting material of the “Arman”, “Zailiyskiy”, and “Dunganskiy” cultivars harbors latent microbial infections, which persist in bulb tissues after prolonged storage and become apparent upon introduction into *in vitro* culture.

It was established that the use of a standard surface sterilization protocol does not ensure complete decontamination of explants, as evidenced by the high levels of contamination observed at early stages of cultivation. The microbial community was dominated by fungi of the genera *Fusarium*, *Penicillium*, and *Aspergillus*, as well as bacteria of the genus *Pseudomonas*, indicating the complex nature of the infection.

The study showed that the level and composition of contamination vary depending on the cultivar, which should be considered when developing protocols for micropropagation and sanitation of garlic planting material.

The obtained results have practical significance for plant biotechnology and highlight the need to improve the preparation of planting material, including optimization of sterilization procedures and implementation of additional decontamination strategies.

Future work should focus on molecular-genetic identification of microorganisms and expansion of the sample size to increase the reliability of the results.

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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: **Jantassov S.K.** — conceptualization, methodology, supervision, formal analysis; **Matai Zh.M.** — investigation, data curation, formal analysis, writing — original draft, conducted *in vitro* experiments, performed data analysis, and draft writing; **Ibragimova G.M.** — validation, writing — review & editing, visualization.

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Сарымсақты (*Allium sativum* L.) *in vitro* дақылына енгізу кезіндегі бактериялық және саңырауқұлақтық контаминация

Зерттеу жұмысында сарымсақты (*Allium sativum* L.) *in vitro* дақылына енгізу барысында туындайтын бактериялық және саңырауқұлақтық контаминация зерттелді. Зерттеудің мақсаты — экспланттардағы контаминация деңгейін бағалау және стандартты беткі стерилизациялау протоколдарының тиімділігін анықтау. Зерттеудің ғылыми жаңалығы — Қазақстанда алғаш рет сарымсақты *in vitro* дақылына енгізу кезінде кездесетін бактериялық және саңырауқұлақтық контаминацияға кешенді бағалау жүргізілді. Тәжірибелер нәтижесінде этанолмен және дезинфекциялаушы ерітінділермен өңдеуді қамтитын дәстүрлі стерилизация әдістері өсімдік материалын толық залалсыздандыруды қамтамасыз етпейтіні анықталды. Соның салдарынан өсірудің бастапқы кезеңдерінде микробтық инфекция дамып, экспланттардың жартылай немесе толық некрозына және микроклональды көбейту тиімділігінің төмендеуіне әкелді. Контаминация деңгейінің сарымсақ сорттарына байланысты өзгеретіні анықталды, бұл донор өсімдіктердің генотипі мен физиологиялық күйінің әсерін көрсетеді. Микроскопиялық талдау нәтижесінде *Fusarium*, *Penicillium*, *Aspergillus* туыстарына жататын саңырауқұлақтардың басым екені, сондай-ақ әртүрлі бактериялық микрофлораның бар екені анықталды. Дегенмен контаминанттарды нақты анықтау үшін қосымша молекулалық-генетикалық зерттеулер жүргізу қажет. Алынған нәтижелер контаминацияның сарымсақты *in vitro* дақылына сәтті енгізудегі негізгі шектеуші факторлардың бірі болып қала беретінін көрсетті. Осыған байланысты өсімдік материалын алдын ала өңдеу әдістерін жетілдіру, сондай-ақ стерилизациялау протоколдары мен өсіру жағдайларын оңтайландыру қажет. Зерттеудің практикалық маңыздылығы — контаминацияны азайтудың тиімді тәсілдерін әзірлеу арқылы микроклональды көбейту тиімділігін арттыру және сау *in vitro* коллекциялар құру мүмкіндігімен сипатталады.

Кілт сөздер: сарымсақ, *Allium sativum* L., *in vitro*, микробтық контаминация, экспланттар, *Fusarium*, *Penicillium*.

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Бактериальная и грибковая контаминация при введении чеснока (*Allium sativum* L.) в культуру *in vitro*

В настоящем исследовании изучалась бактериальная и грибная контаминация, возникающая при введении чеснока (*Allium sativum* L.) в культуру *in vitro*. Целью работы являлась оценка уровня контаминации эксплантов, а также определение эффективности стандартных протоколов поверхностной стерилизации. Научная новизна исследования заключается в том, что впервые в Казахстане проведена

комплексная оценка бактериальной и грибной контаминации при введении чеснока в культуру *in vitro*. В ходе экспериментов установлено, что традиционные методы стерилизации, включая обработку этанолом и дезинфицирующими растворами, не обеспечивают полной деконтаминации растительного материала. В результате на ранних этапах культивирования наблюдалось развитие микробной инфекции, приводящее к частичному или полному некрозу эксплантов и снижению эффективности микроклонального размножения. Установлено, что степень контаминации варьирует в зависимости от сорта чеснока, что свидетельствует о влиянии генотипа и физиологического состояния донорных растений. Микроскопический анализ выявил преобладание грибов родов *Fusarium*, *Penicillium* и *Aspergillus*, а также наличие разнообразной бактериальной микрофлоры. Однако точная идентификация контаминантов требует проведения дополнительных молекулярно-генетических исследований. Полученные результаты показывают, что контаминация остаётся одним из основных ограничивающих факторов успешного введения чеснока в культуру *in vitro*. В связи с этим необходима оптимизация предпосевной обработки растительного материала, а также совершенствование протоколов стерилизации и условий культивирования. Практическая значимость исследования заключается в разработке более эффективных подходов к снижению контаминации, что позволит повысить эффективность микроклонального размножения и создать здоровые *in vitro* коллекции.

Ключевые слова: чеснок, *Allium sativum* L., *in vitro*, микробная контаминация, экспланты, *Fusarium*, *Penicillium*.

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