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Original article

CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF CAUSAL AGENTS OF *CYTOSPORA* CANKER OF APPLE TREES IN KAZAKHSTAN

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ABSTRACT

This study provides a morphological description of *Cytospora* spp. isolates collected from apple tree branches affected by Cytospora canker. Cytospora canker is a globally widespread disease caused by highly aggressive pathogens that induce cankers in woody plants within both natural and agro-ecosystems. In severe cases, cankers can lead to the dieback of branches and trees. This article presents the first report of *Cytospora sorbina* and *Cytospora parasitica* species identified in intensive apple orchards of Almaty region, Kazakhstan. Notably, the species composition, as well as the morphological and genetic characteristics of Cytospora spp. pathogens causing Cytospora canker in Kazakhstan, remain understudied. Consequently, this study examined the cultural and morphological properties of Cytospora canker pathogens, including determining optimal growth media and temperature conditions for these pathogens. For microbiological analysis, samples were collected from five intensive apple orchards in the Almaty region, yielding 15 fungal isolates from the following apple varieties: Gala, Granny Smith, Golden Delicious, Golden Rush and Fuji. Based on morphological and cultural characteristics, the isolates were identified as *Cytospora sorbina* and *Cytospora parasitica*. The results showed optimal growth conditions at 27°C on potato dextrose agar (PDA). This study provides insights into the host-pathogen biological interactions, potentially facilitating improvements in early diagnostics and the development of integrated disease management strategies against Cytospora-related diseases to protect apple orchards.

Keywords: *apple, apple diseases, Cytospora canker, Cytospora spp., microbiological analysis, pycnidia.*

1 INTRODUCTION

Apple production is a significant contributor to Kazakhstan's agricultural sector, playing an essential role in supporting the nation's economic stability. Kazakhstan has the potential to use apple production to enter international markets, thereby promoting international trade relationships and attracting foreign investments in the agricultural sector [1].

According to the Food and Agriculture Organization (FAO), in 2022, Kazakhstan's apple planting area totaled 35,727 hectares, with a yield of 74,990 tons/hectare and an annual apple production of 267,919 tons. Despite the favorable climatic conditions in Kazakhstan's southern regions, apple orchard productivity has declined in recent years due to various fungal diseases [2].

In a comprehensive survey of fungal diseases affecting apple trees in southern and southeastern Kazakhstan, researchers Ismailova E.T., Sadanov A.K., and others identified moniliasis, scab, rust, leaf spots, and Cytospora canker as common diseases in these areas, with Cytospora canker found across all surveyed regions, albeit at varying levels of severity [3]. Prior studies by Ospanova A. et al. documented the presence of Cytospora canker in central Kazakhstan (Karaganda, Astana) on various trees, characterizing the phytopathogens by morphological features [4].

Today, fungal diseases pose a severe and growing threat to apple orchards in Almaty region. In recent years, orchard phytosanitary conditions have deteriorated due to climate change and nutrient deficiencies, increasing trees' susceptibility to Cytospora canker pathogens (personal communication with apple producers).

Many trees suffer extensive damage from Cytospora canker lesions, which form on the trunk, major limbs, and branches [5, 6]. Trees are particularly vulnerable to the disease when grown under stressful conditions, such as on poor sites or following drought or frost events [7].

Cytospora canker is not immediately visible; affected trunks and branches show bark dieback, which gradually turns brown and is bordered by roughened tissue. Later, the bark peels away, revealing necrotic tissue beneath, where small pycnidia appear, resembling ulcers. Conidia from the pycnidia may ooze out during wet weather, allowing the disease to spread to nearby healthy trees [8, 9]. Most infections occur in late autumn, early winter, or late winter/early spring, although weakened trees may be infected throughout the growing season. Over the season, mycelium and conidia develop on infected tree parts [10-12].

Pathogen conidia enter small and large branches through natural or mechanical wounds. The fungus reproduces in dead tissue and spreads to adjacent live tissues, forming cankers on the trees. These pathogens colonize and obstruct the vascular system, impeding water transport to infected trunks, shoots,

and branches, which can ultimately kill individual trees or even entire orchards [13-15].

Cytospora canker in apple has been the subject of extensive research worldwide, yet studies on this disease in Kazakhstan, the origin of the first apple trees, remain limited. This study aims to investigate the Cytospora canker pathogens responsible for the dieback of apple trees in Almaty region orchards.

2 MATERIAL AND METHODS

2.1 Field Sampling and pathogen isolation. The study was conducted to isolate *Cytospora* spp. fungi from apple trees exhibiting typical canker symptoms in major intensive orchards in the Almaty region. Field surveys were carried out to identify symptomatic trees, from which branches were collected for laboratory analysis. The sampling focused on trees with prominent signs of Cytospora canker. The collected branches were then processed to obtain fungal isolates for further study.

2.2 Microbiological analysis. To isolate the pathogens, pieces of bark containing dark pycnidial stromata were excised from affected branches. These samples were placed on nutrient media to promote fungal growth and isolate the pathogens. The media used included potato dextrose agar (PDA), Sabouraud chloramphenicol dextrose agar (SCA), and Czapek Dox agar (CDA), prepared as follows:

- PDA (Potato Dextrose Agar): dextrose 20.000 g/L, agar 15.000 g/L, potato extract 4.000 g/L, pH 5.6 ± 0.2.

- SCA (Sabouraud Chloramphenicol Dextrose Agar): dextrose 40.000 g/L, agar 15.000 g/L, peptone 10.000 g/L, chloramphenicol 0.5 g/L, pH 5.6 ± 0.2.

- CDA (Czapek Dox Agar): sucrose 30.000 g/L, agar 15.000 g/L, $NaNO_3$ 2.000 g/L, K_2HPO_4 1.000 g/L, $MgSO_4$ 0.500 g/L, KCl 0.500 g/L, $FeSO_4$ 0.010 g/L, pH 7.3 ± 0.2.

Colonies of interest were subcultured to obtain pure fungal isolates for detailed characterization.

2.3 *Morphological and microscopic analysis.* The cultural and morphological characteristics of *Cytospora* spp. isolates were observed, focusing on pycnidia formation, stromata shape and color, and conidia morphology. The macromorphological characteristics of colonies were studied by recording measurements on days 5-7-14 of growth. Microscopic examination was performed using a ZEISS Axio Scope.A1 (Germany) microscope to capture detailed micromorphological features of the isolates.

3 RESULTS

The survey of major apple orchard production zones (Almaty region) revealed that Cytospora canker is widespread. Across the five intensive orchards examined, Cytospora canker was present in each, with one orchard showing an exceptionally high incidence rate. The apple varieties assessed included Gala, Granny Smith, Golden Delicious, Pink Lady, Golden Rush, Enterprise, Fuji, Maksat, Star Crimson, and Red Delicious.

Referring to studies by Chinese researchers (Xiaojie, Liu & Li, Xiao-Shuang & Bozorov et al.), who described and identified six species of Cytospora spp. causing canker in apple trees, we can confirm that our isolated cultures correspond to this genus of fungi [8, 9].

Upon visual inspection of the samples, typical symptoms of Cytospora canker in apple trees were observed: desiccation of young shoot tips, leaf wilting, and darkened bark areas with circular cankers (Figure 1).

Pycnidial conidiomata, embedded in the bark, emerge through the bark surface at maturity, with multiple nests (Figure 1, A-C).

To isolate the pathogen, 32 samples were collected, of which 15 displayed cultural characteristics consistent with Cytospora spp. Fungal growth was monitored on culture media for 7-14 days, with optimal growth observed on PDA medium at 27°C (Figures 2 and 3).

Cultures of *Cytospora sorbina* on PDA medium (Figure 2): rapid-growing colonies with whitish mycelium (day 5), later darkening to light brown or dark yellow. Colonies have a rough texture, with uneven edges and a homogeneous consistency (day 7).

Cultures of *Cytospora sorbina* on Sabouraud medium: moderately growing colonies with a yellowish hue (day 5), later expanding but halting growth by day 7, with no further increase in size or change in color. Colonies are fluffy with irregular, torn edges and non-uniform consistency.

Cultures of *Cytospora sorbina* on Czapek medium:

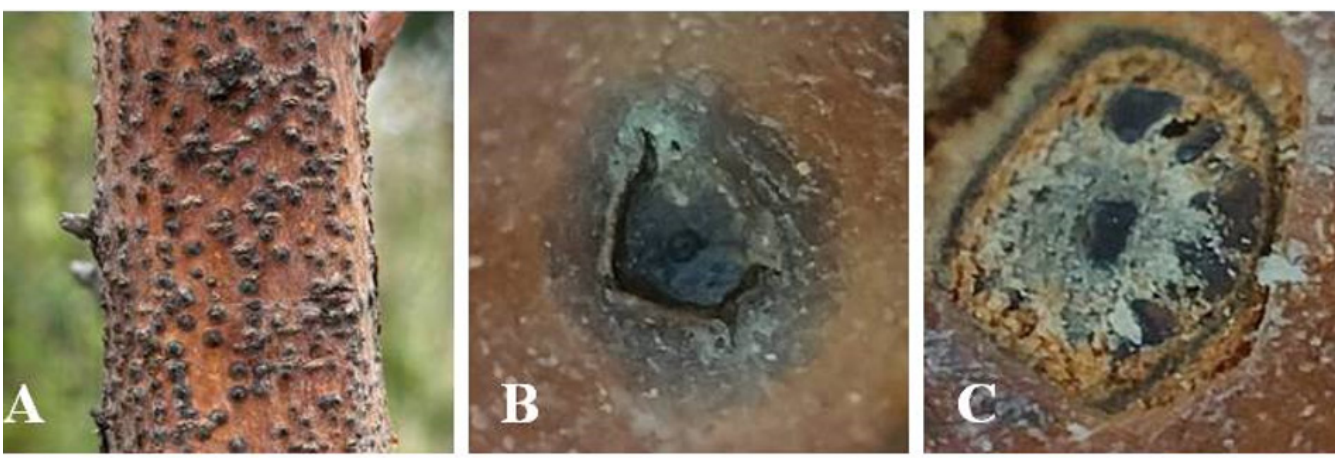

Figure 1 – Disease symptoms caused by *Cytospora* species on apple trees in Almaty region, Kazakhstan

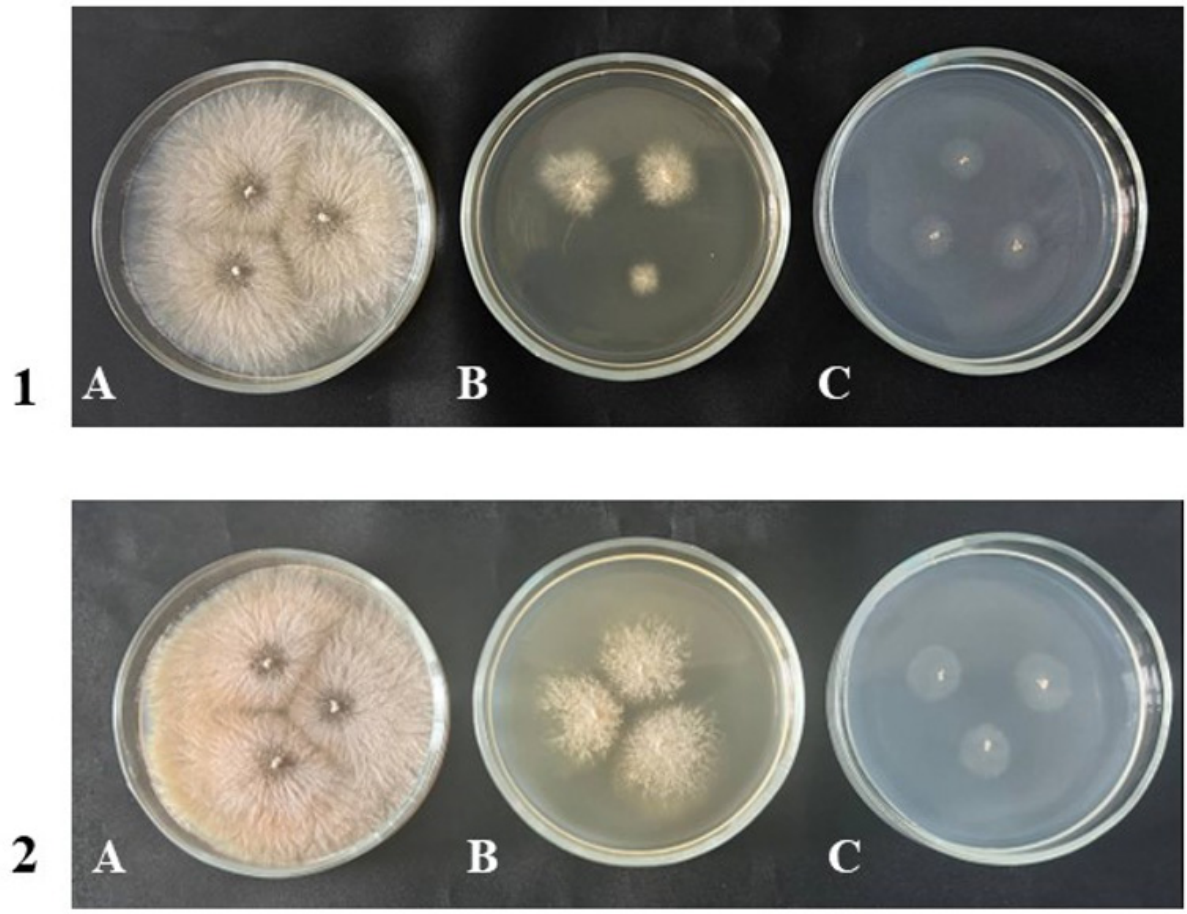

Figure 2 – Cultural characteristics of *Cytospora sorbina*: 1 – culture growth on day 5, 2 – culture growth on day 8, A – growth on PDA medium, B – growth on Sabouraud medium, C – growth on Czapek medium.

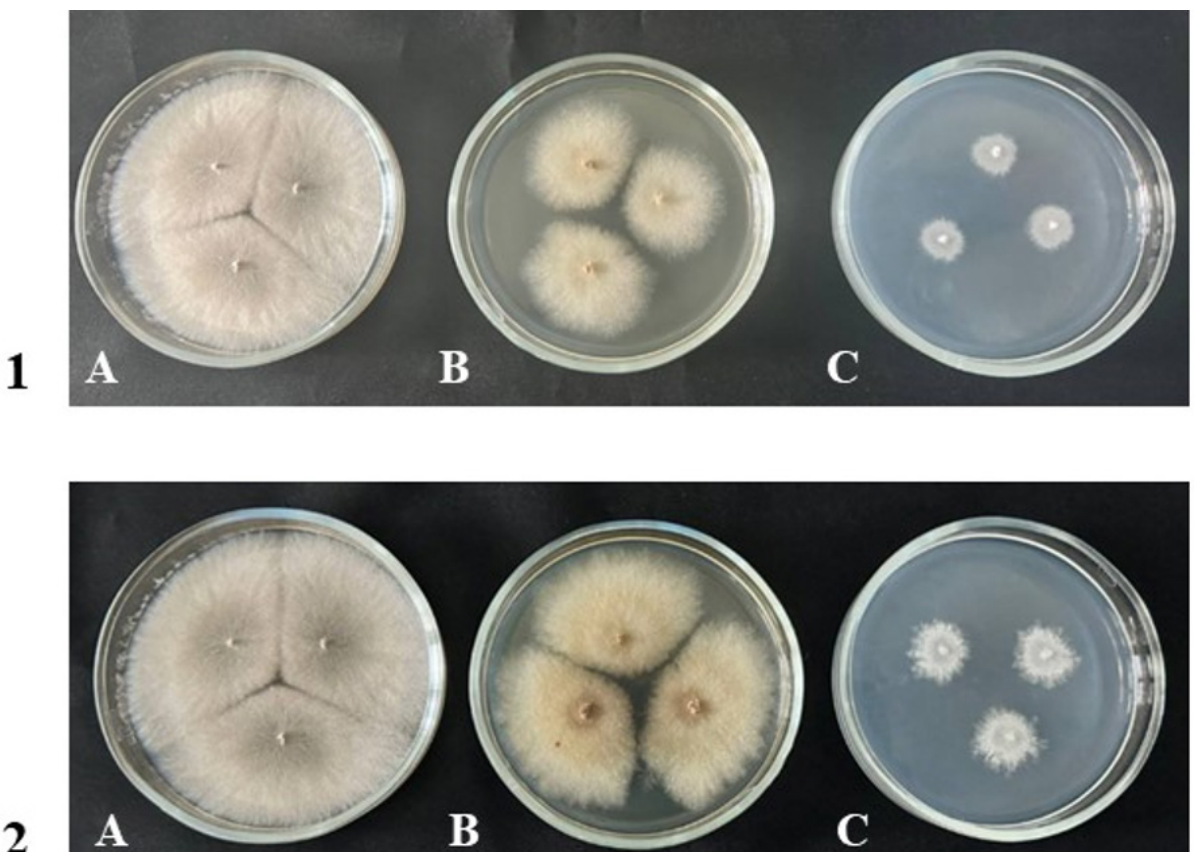

Figure 3 – Cultural characteristics of *Cytospora parasitica*: 1 – culture growth on day 5, 2 – culture growth on day 8, A – growth on PDA medium, B – growth on Sabouraud medium, C – growth on Czapek medium.

slow-growing, colorless, nearly transparent colonies (day 5), later growing without significant changes in colony size (day 7), while retaining color and consistency. Colonies are flat with smooth edges and a homogeneous consistency.

Cultures of *Cytospora parasitica* on PDA medium (Figure 3): rapid-growing colonies with fluffy, grayish-white aerial mycelium (day 5), later darkening in the center to dark gray, while colony edges remain unchanged. Colonies are aerial with smooth edges and homogeneous consistency (day 7).

Cultures of *Cytospora parasitica* on Sabouraud medium: rapidly growing colonies with white aerial mycelium (day 5), later darkening to a yellowish or light brown. Colonies are fluffy with uneven edges and homogeneous consistency (day 7).

Cultures of *Cytospora parasitica* on Czapek medium: slow-growing white colonies (day 5), with dense structure, irregular edges, and homogeneous consistency (day 7).

Due to differences in growth rates and patterns on the three different media, weakly alkaline media are considered preferable for cultivating *Cytospora* spp. Neutral media pH and the presence of salts inhibited pathogen growth, while disaccharides slowed growth, and monosaccharides accelerated it. The presence of potato extracts positively influenced growth.

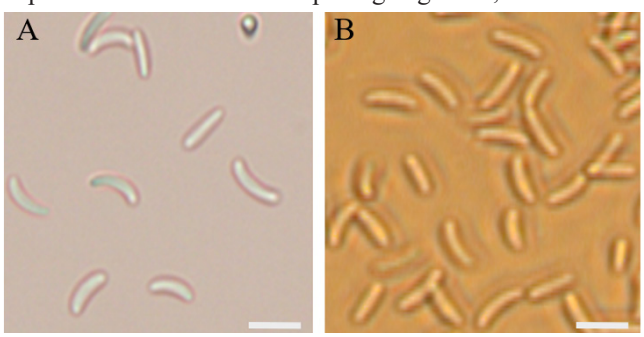

Figure 4 – Morphological structures of phytopathogens: conidia (×100, SB 10 µm.) A – *Cytospora sorbina*, B – *Cytospora parasitica*

Conidia (Figure 4) are smooth-walled, elongated-allantoid, and aseptate. Sexual morph was not observed.

The isolates identified as *Cytospora sorbina* and *Cytospora parasitica*, which were previously determined by us in earlier studies, have been deposited in the GenBank database [16, 17].

Microbiological analysis of the selected samples revealed isolates from apple varieties Gala, Granny Smith, Golden Delicious, Golden Rush and Fuji, which were identified by morphological, cultural, and molecular genetic analysis as *Cytospora sorbina* and *Cytospora parasitica*. These varieties showed greater susceptibility to cytospora canker than other varieties studied.

4 DISCUSSION

The findings of this study mark a significant step forward in understanding the causative agents of canker and dieback diseases in apple orchards within the Almaty region of Kazakhstan. The discovery of *C. parasitica* and *C. sorbina* as key pathogens not only expands the known geographical distribution of these species but also underscores the potential risks associated with the introduction of new apple cultivars and changing environmental conditions.

Previous studies have reported *C. parasitica* and *C. Sorbina* in neighboring countries such as China [13], Iran [5], and Turkey [14], suggesting a possible connection between its spread and regional environmental or trade-related factors. However, the detection of *C. sorbina* in this study raises new questions about the adaptability of *Cytospora* species to different climatic zones. These findings contribute to the growing body of evidence that *Cytospora* species are more influenced by environmental factors than host specificity, as highlighted by Pan et al. [7].

Despite these advances, there are limitations to this study. Future research should focus conducting pathogenicity tests on healthy branches is necessary to fully capture the complexity of host-pathogen interactions in natural conditions. The genetic diversity of *C. parasitica* and *C. sorbina* populations in Kazakhstan remains unexplored, which could provide insights into their evolutionary adaptations.

Integrating these findings into the current understanding of apple canker disease emphasizes the need for regional surveillance programs, which is a recognized hotspot for Cytospora infections.

This study opens new avenues for future research. For instance, comparative studies on the pathogenicity mechanisms of *C. parasitica* and *C. sorbina* could reveal why these species exhibit varying levels of virulence. Exploring the role of environmental factors, such as temperature fluctuations and water stress, in influencing disease severity may also provide valuable insights. Additionally, investigating potential biocontrol agents or resistant apple cultivars could contribute to developing sustainable management strategies.

By shedding light on previously undocumented pathogens in Kazakhstan, this research provides a foundation for future epidemiological studies and reinforces the importance of proactive disease management to safeguard apple production in the region.

CONCLUSION

As a result of the survey conducted in apple orchards in the Almaty region, it was found that Cytospora cancer has become widely spread. Trees affected by *Cytospora spp.* were identified in all surveyed orchards.

Microbiological analysis of the collected samples resulted in the isolation of 15 fungal strains from the following apple varieties: Gala, Granny Smith, Golden Delicious, Golden Rush and Fuji. Based on morphological and cultural characteristics, these strains were identified as *Cytospora sorbina* and *Cytospora parasitica*. Molecular genetic identification of the strains was carried out, with the most optimal growth observed using PDA medium.

Thus, this study was initiated to identify the *Cytospora spp.* species responsible for apple canker and dieback diseases in intensive orchards of the Almaty region using a cultural-morphological approach.

This article provides research findings on Cytospora canker in fruit crops, shedding light on current issues and future opportunities for improving disease control methods.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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КУЛЬТУРАЛЬНО-МОРФОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА ВОЗБУДИТЕЛЕЙ ЦИТОСПОРОЗА ЯБЛОНЬ В КАЗАХСТАНЕ

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АННОТАЦИЯ

В данном исследовании было проведено морфологическое описание изолятов *Cytospora* spp., которые были выделены из пораженных цитоспорозом ветвей яблони. Цитоспороз – распространенное по всему миру заболевание, возбудители которых представляют собой агрессивных патогенов, вызывающих язвы древесных растений, как в природ-

ных, так и в агроэкосистемах по всему миру. В тяжелых случаях язвы могут привести к отмиранию ветвей и деревьев. В данной статье представлено первое сообщение о видах *Cytospora sorbina и Cytospora parasitica*, обнаруженных на территории интенсивных яблоневых садов Алматинской области, Казахстан. В частности, видовая принадлежность и морфологические/генетические особенности патогенов *Cytospora* spp., вызывающих цитоспорозный рак яблонь на территории Казахстана мало изучены. В связи с этим, в данном исследовании были изучены культурально-морфологические свойства возбудителей цитоспороза, а также подобрана оптимальная среда и температурные условия для роста возбудителей. В результате исследования проведен отбор биоматериала для микробиологического анализа с 5 интенсивных яблоневых садов Алматинской области, выделено 15 грибных изолятов из следующих сортов яблонь: Gala, Granny Smith, Golden Delicious, Golden Rush and Fuji, которые по морфологическим и культуральным признакам, соответствовали *Cytospora sorbina и Cytospora parasitic*a. Результаты показали, что оптимальные условия для роста наблюдались при 27°C на среде PDA (potato dextrose agar). Данное исследование поможет понять биологические особенности взаимодействия между хозяином и патогеном, для способствования в улучшении ранней диагностики и правильного подбора интегрированных стратегий борьбы с болезнями, вызванной грибами данного рода для сохранения садов.

Ключевые слова: *яблоня, болезни яблонь, цитоспорозный рак, Cytospora spp., микробиологический анализ, пикнидии.*

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ҚАЗАҚСТАНДАҒЫ АЛМА АҒАШТАРЫНЫҢ ЦИТОСПОРОЗ ҚОЗДЫРҒЫШТАРЫНЫҢ ДАҚЫЛДЫҚ-МОРФОЛОГИЯЛЫҚ СИПАТТАМАСЫ

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АНДАТПА

Бұл зерттеуде цитоспорамен зақымдалған алма ағашының бұтақтарынан бөлінген *Cytospora* spp изоляттарының морфологиялық сипаттамасы жүргізілді. Цитоспороз – бүкіл әлемдегі табиғи және агроэкожүйедегі ағаш өсімдіктерінде ісік тудыратын агрессивті патогендерден туындаған дүниежүзілік ауру. Ауыр жағдайларда ісіктер бұтақтар мен ағаштардың өлуіне әкелуі мүмкін. Бұл мақалада Қазақстанның Алматы облысындағы қарқынды алма бақтарында кездесетін *Cytospora sorbina* және *Cytospora parasitica* түрлеріне алғашқы рет сипаттама берілген. Атап айтқанда, Қазақстанда алма ағаштарының цитоспора ісігін тудыратын Cytospora spp. қоздырғыштарының түрлері мен морфологиялық/генетикалық ерекшеліктері аз зерттелген. Осыған байланысты бұл зерттеуде цитоспора қоздырғыштарының дақылдық және морфологиялық қасиеттері зерттеліп, қоздырғыштардың көбеюіне қолайлы орта мен температуралық жағдайлар оңтайланды. Зерттеу нәтижесінде Алматы облысындағы 5 интенсивті алма бақтарынан микробиологиялық талдау үшін биоматериал іріктелді, алма ағаштарының келесі сорттарынан 15 саңырауқұлақ изоляттары бөлініп алынды: Gala, Granny Smith, Golden Delicious, Golden Rush, Fuji. Олар морфологиялық және дақылдық белгілері бойынша *Cytospora sorbina* және *Cytospora parasitica* түрлеріне сәйкес келеді. Нәтижесінде PDA (potato dextrose agar) қоректік ортада 27°C температурада оңтайлы өсу жағдайлары байқалғанын көрсетті. Бұл зерттеу иесі мен қоздырғыштың өзара әрекеттесуінің биологиялық ерекшеліктерін түсінуге, ерте диагностиканы жақсартуға және бақтарды сақтау үшін осы тектес саңырауқұлақтар тудыратын аурулармен күресудің кешенді стратегиясын дұрыс таңдауға ықпал етеді.

Түйін сөздер: *алма ағашы, алма ағашының аурулары, цитоспора ісігі, Cytospora spp., микробиологиялық талдау, пикнидиялар.*