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CIRSIUM ARVENSE – AS A NATURAL RESERVOIR OF PHYTOPATHOGENIC FUNGI

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ABSTRACT

Cirsium arvense is among the most pervasive and resilient weed species, playing a critical role in the epidemiology of phytopathogenic fungi within agroecosystems. The present study aimed to characterize fungal pathogens associated with *C. arvense* populations growing adjacent to cereal and legume crop fields in the North Kazakhstan region. Fungal isolates were obtained from symptomatic plant tissues, including leaves, stems, and inflorescences. Pure cultures were established through cultivation on nutrient media under controlled conditions. Molecular identification was conducted using ribosomal DNA internal transcribed spacer (ITS) region sequencing, and species-level classification was achieved by comparison with GenBank entries via BLAST analysis. The dominant fungal taxa identified were *Alternaria alternata* and *Fusarium tricinctum*. To elucidate the evolutionary placement of the isolates, a phylogenetic tree was constructed using the Neighbor-Joining method implemented in MEGA 11 software. The findings indicate that C. arvense functions as a natural reservoir for economically important phytopathogenic fungi, underscoring its epidemiological relevance and the need for integrated weed and disease management strategies in agricultural systems.

Key words: *Cirsium arvense, phytopathogenic fungi, ITS identification, Alternaria alternata, Fusarium tricinctum*

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1 INTRODUCTION

Cirsium arvense, commonly known as Canada thistle, is considered one of the most aggressive weed species, widely distributed across agricultural lands in Europe, Asia, and North America [1]. Under conditions of monoculture and minimal soil disturbance, particularly on organic farms, this species demonstrates high competitiveness and resistance to herbicidal treatment. From an ecological standpoint, *C. arvense* not only negatively impacts crop yields but also poses a phytosanitary risk as a potential natural reservoir of phytopathogenic fungi [2].

Fungal genera commonly associated with *C. arvense* include *Alternaria*, *Fusarium*, *Phoma*, *Paraphoma*, *Puccinia*, and others. Particular attention is given to *Alternaria alternata*, a widely distributed pathogen identified both on *C. arvense* leaves and on cultivated plants such as potato. *Fusarium* species, frequently isolated from weed hosts, exhibit a strong ability to overwinter and preserve inoculum between growing seasons, thereby contributing to the infection of agricultural crops [3, 4, 5, 6].

Some fungi isolated from *C. arvense*, such as *Paraphoma* sp., have demonstrated pronounced phytotoxic properties and are being considered as potential bioherbicides. Moreover, the obligate biotroph *Puccinia punctiformis* remains a promising agent for the biological control of *C. arvense*. Due to its high host specificity and efficacy, this pathogen is viewed as a preferred candidate for biocontrol; however, large-scale production of viable spores remains a significant challenge [7].

In the context of increasing weed resistance to synthetic herbicides and the development of organic farming, biological control using fungal agents is gaining growing importance [8]. An integrated approach that includes the study of weed-associated mycobiota allows for both accurate assessment of phytosanitary risks and the discovery of novel biocontrol agents in resilient agroecosystems [9, 10].

In Kazakhstan, insufficient attention has been paid to weedy plant species growing near cultivated fields. The health, resilience, and productivity of crops depend not only on direct cultivation practices, but also on the composition of surrounding weed flora. Understanding the diversity of wild plants and their associated pathogens may provide a foundation for preventing crop diseases and pests, thereby helping agricultural enterprises avoid significant economic losses [11].

The aim of this study is to evaluate the capacity of *C. arvense* to accumulate major fungal pathogens of agricultural crops and to highlight its role as a reservoir of phytopathogenic strains.

2 MATERIALS AND METHODS

2.1 Isolation of fungal pathogens from *Cirsium arvense*

Vegetative material of the *Cirsium arvense* plant (n=65) was collected in the North Kazakhstan region within agri-

cultural lands adjacent to grain crops. The samples included above-ground plant organs with signs of damage by phytopathogenic fungi and were used for subsequent mycological analysis. Individual parts of the *Cirsium arvense* (leaves, flowers, stem) are first washed under running water for 1-2 hours, then disinfected with 96% alcohol for 1-2 minutes. After disinfection, the vegetative material is rinsed with sterile water and dried between layers of sterile filter paper. Small pieces are placed in a Petri dish with a nutrient medium and incubated in a thermostat at a temperature of 25–27 °C for germination, which usually lasts 5-7 days.

2.2 DNA extraction and molecular characterization

Genomic DNA was extracted from fungal strain using liquid nitrogen and phenol-chloroform extraction method, and the genomic DNA was analyzed by electrophoresis on 1% agarose gel. The *ITS* region on rDNA was amplified by using specific primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) (Integrated DNA Technologies, Inc., USA). The PCR reaction was done in a SimpliAmp thermal cycler (Applied biosystems) under the following conditions: an initial denaturation set up at 94 °C for 5 min was followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 52 °C for 40 sec and extension at 72 °C for 50 sec, with a final extension step of 72 °C for 7 min.

2.3 PCR samples purification

PCR samples were purified from oligonucleotide residues by dephosphorylation using alkaline phosphatase (SAP - shrimp alkaline phosphatase) and endonuclease. A mixture was prepared in a total volume of 10 µl for each sample - dH2O - 7.25 µL, 10× PCR Buffer - 1.0 µl, MgCl2 - 1.0 µl, SAP (5 mM) - 2.5 µl, Exonuclease I (5 units/µL) - 0.125 µl. The resulting mixture was added to each PCR product, placed in a thermal cycler under the following conditions: 37°C - 30 min, 85°C - 15 min, 4°C - ∞. Sample preparation for sequencing carried out by precipitation with an alcohol-acetate mixture.

2.4 DNA sequencing

The components of a standard set of reagents for the sequencing reaction were prepared in a 0.2-ml thin-walled thermocycler tube. A standard set of reagents for cyclic sequencing using *CEQ WellRED* terminator dyes (partially mixed). The following thermal cycle program was chosen: 96°C - 20 sec, 50°C - 20 sec, 60°C - 4 min for 30 cycles and followed by aging at 4°C. The sequencing was done by using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and the sequence was deposited in GenBank. These sequences were compared with other sequences in the GenBank by using the BLAST analysis. The phylogenetic analysis was carried out with MEGA 11 software.

2.5 Statistical analysis

This study used three main indicators of vegetative mate-

Figure 1 – Fungal lesions on *Cirsium arvense*: A - Growth in the field, B – leaves, C – flowers, D – stem.

rial: leaves, stems, flowers, which are essentially indicators of infection with phytopathogenic fungi. Differences in the prevalence of infections among different parts of vegetative material and pathogen species in percentage. All statistical analyses were performed using Statistix 10 (analytical software).

3 RESULTS

The study was conducted on agricultural lands to identify foci of fungal phytopathogens affecting grain crops. The seed material was treated with fungicidal preparations both before sowing and during the growing season. Despite the protective measures taken, foci of phytopathogenic fungi continued to form. An analysis of the phytosanitary situation showed that the main source of inoculum were weeds growing along the roadsides adjacent to agricultural lands. In particular, it was found that *Cirsium arvense* (Figure 1) is a localization of phytopathogenic fungi capable of infecting crops.

Foci of fungal pathogens were visually determined on *Cirsium arvense* plants. For mycological analysis, areas with the most pronounced symptoms of the disease were selected, including leaves, flower structures and stems. The material was seeded on nutrient media to isolate and cultivate pathogens. Incubation was carried out under optimal temperature conditions for 5-7 days. Upon completion of the incubation period, the main fungal phytopathogens associated with the affected tissues were isolated Figure 2).

Microscopic analysis of fungal isolates revealed characteristic morphological features. In *Alternaria* spp. the hyphae are septate, light to dark brown, smooth-walled, and branched. The conidiophores are straight or slightly flexuous, often with a swollen base, and dark brown in color. Conidia are arranged in chains, darkly pigmented, and vary from ellipsoid to pyriform or obclavate in shape. They possess both transverse and longitudinal septa, forming so-called dictyospores. A distinctive apical beak is commonly present, which serves as a key diagnostic feature of the species. The size of the conidia typically ranges from 20–60×7–18 µm [12, 13, 14].

Fusarium spp. produces hyaline, septate hyphae with smooth walls. Both microconidia and macroconidia are formed in culture. Microconidia are oval to fusiform, single- or two-celled, often aggregated in false heads on short conidiophores. Macroconidia are characteristically falcate (sick-

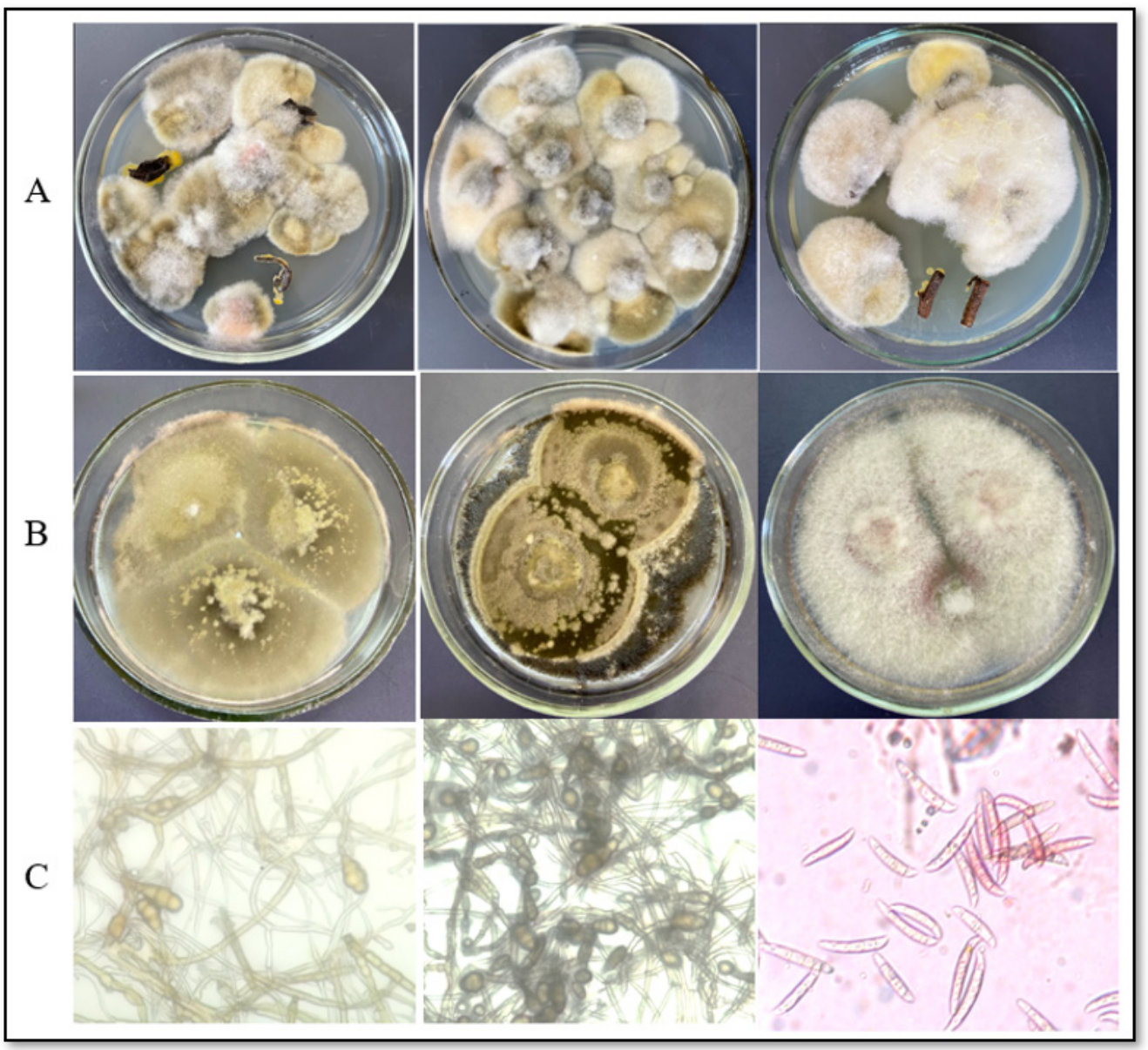

Figure 2 – Growth of fungal pathogens: A – Primary seeding; B – Pure isolates C - Strain microscopy

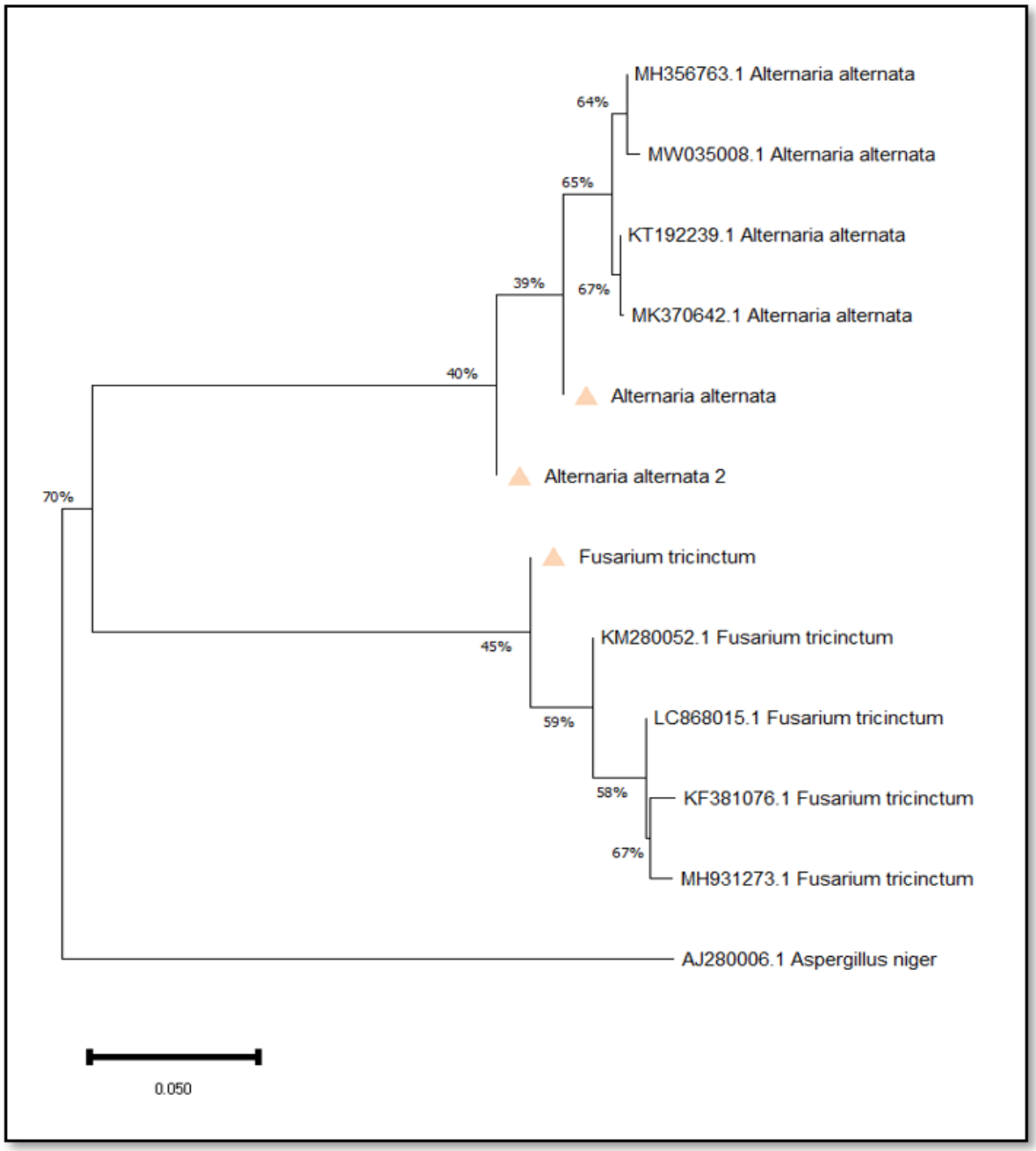

Figure 3 – Phylogenetic tree obtained by the Neighbor-Joining method.

le-shaped), curved, with 3 to 5 septa, and tapered at both ends; they measure approximately 25–60×3-5 µm. In older cultures, chlamydospores can be observed as round, thick-walled cells, either singly or in chains. Colonies on potato dextrose agar (PDA) typically exhibit a fluffy or cottony texture and show coloration ranging from pink to cream or yellowish tones [15].

Genomic DNA was isolated from the resulting pure fungal cultures using the standard CTAB (cetyltrimethylammonium bromide) method, followed by purification by phenol-chloroform extraction. For molecular genetic identification of fungal isolates, the internal transcribed spacer (*ITS* region) of ribosomal DNA was amplified using the universal primers *ITS1* and *ITS4*. The amplified fragments were sequenced and the resulting sequences were compared with the NCBI GenBank database using the BLAST algorithm to determine the species affiliation of the isolates.

Based on the results of molecular genetic identification based on the analysis of the *ITS* regions of rDNA, it was established that the studied fungal isolates belong to the species *Alternaria alternata* and *Fusarium tricinctum*. The obtained nucleotide sequences were used to construct a phylogenetic tree in the MEGA 11 software using the Neighbor-Joining method [16]. This analysis allowed us to determine the phylogenetic position of the isolates and identify their evolutionary relationships with the taxa presented in the GenBank database (Figure 3).

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [17] and are in the units of the number of base substitutions per site. The proportion of sites where at least 1 unambiguous base is present in

Table 1 - Frequency of fungal pathogen isolation from different organs of *Cirsium arvense.*

Plant Organ	Number of Samples	Alternaria alternata, n (%)	Fusarium tricinctum, n (%)	Other Isolates, n (%)
Leaves	30	18 (60%)	10 (33%)	4 (13%)
Stems	20	8 (40%)	7 (35%)	3 (15%)
Inflorescences	15	4 (27%)	5 (33%)	2 (13%)
Total	65	30 (46%)	22 (34%)	9 (14%)

at least 1 sequence for each descendent clade is shown next to each internal node in the tree. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA11 [18]

To assess the distribution of fungal pathogens across different plant organs of *Cirsium arvense*, a total of 65 samples were analyzed. The plant material included leaves, stems, and inflorescences with visible signs of fungal infection. From these samples, fungal isolates were cultivated and identified based on morphological features and DNA analysis. The results of the pathogen frequency by plant organ are summarized in Table 1.

As shown in Table 1, *Alternaria alternata* was the most frequently isolated pathogen, particularly from leaf tissues, with a detection rate of 60%. *Fusarium tricinctum* was found across all examined organs, with relatively consistent frequencies ranging from 33% to 35%. A smaller proportion of samples contained other fungal isolates, including unidentified saprophytes and secondary colonizers. These results highlight the importance of *C.arvense* as a reservoir for phytopathogenic fungi, especially in leaf tissues, which may act as a primary inoculum source for neighboring crops.

4 DISCUSSION

Cirsium arvense is a perennial, clonal weed species native to Europe and Western Asia but now widely distributed across temperate regions of the Northern Hemisphere. It is considered one of the most aggressive invasive weeds in North America, Central Asia, and parts of Australia due to its high reproductive capacity and resistance to conventional control methods [19, 20]. In Kazakhstan, C. arvense is widely present in agricultural landscapes, especially in northern regions such as North Kazakhstan, Kostanay, and Akmola, where it grows along field borders, roadsides, and uncultivated lands.

Globally, *C. arvense* is known to harbor a variety of phytopathogenic fungi, including species of *Alternaria*, *Fusarium*, *Phoma*, *Paraphoma*, *Cladosporium*, and rust fungi like *Puccinia punctiformis* [21]. These fungi are either endophytic or necrotrophic, contributing to the role of C. arvense as a pathogen reservoir.

In our study, *Alternaria alternata* and *Fusarium tricinctum* were isolated from *C. arvense* samples collected in North Kazakhstan. This corresponds with global reports that identify these fungi as prevalent in Cirsium-associated communities. For example, *A. alternata* is a cosmopolitan pathogen found on numerous crops, including potato, wheat, and sunflower [22]. *F. tricinctum* is part of the Fusarium complex causing root, stem, and head rot in cereals [23]. Therefore, the pathogens found in Kazakhstan align with global trends, confirming that C. arvense plays a similar ecological role in local agroecosystems.

Microscopically, the morphology of the isolated A. alternata conidia – dark, muriform, with transverse and longitudinal septa, and an apical beak – is fully consistent with previous characterizations [24]. Similarly, F. tricinctum exhibited curved, falcate macroconidia with 3-5 septa, as well as oval microconidia and chlamydospores in aging cultures, matching descriptions by [25]. These findings support the reliability of morphological identification and confirm that the strains found in North Kazakhstan do not differ significantly in microstructural features from those described in other geographic locations.

Molecular genetic analysis of the ITS region confirmed the identity of the isolates as *Alternaria alternata* and *Fusarium tricinctum*, with high sequence similarity to reference strains in GenBank. Phylogenetic reconstruction using the Neighbor-Joining method in MEGA 11 showed clustering of the isolates with corresponding species clades, indicating high genetic homology. These results are consistent with previous studies that used ITS-based barcoding for identification of these fungi [26, 27]. The use of ITS markers proved effective for species-level discrimination and supports the taxonomic placement of our isolates within globally recognized lineages.

The results of this study confirm the role of *Cirsium arvense* as a significant phytosanitary factor in agroecosystems. The isolation of *Alternaria alternata* and *Fusarium tricinctum* underscores the potential for long-term inoculum persistence on weedy plants, which poses a risk of reinfection to nearby crops even when seed treatments with fungicides are applied. These findings are in line with previous research highlighting the function of weeds as pathogen reservoirs, particularly in systems with reduced tillage and monoculture practices [28].

It is important to note that the identified phytopathogens are polyphagous and capable of infecting a wide range of economically important crops, ultimately reducing their yield and quality. Therefore, weed control along field margins should be considered an integral component of comprehensive crop protection strategies.

5 CONCLUSION

The conducted study demonstrated that *Cirsium arvense*, growing along the borders of agricultural lands, can serve as a stable natural reservoir of fungal phytopathogens. The identification of *Alternaria alternata* and *Fusarium tricinctum* based on ITS analysis, along with the construction of a phylogenetic tree, confirmed their close genetic relationship with pathogenic strains affecting cultivated crops. These findings emphasize the importance of phytosanitary monitoring not only of crop fields but also of the surrounding weed vegetation to ensure effective prevention of plant diseases in agricultural systems.

AUTHOR CONTRIBUTIONS

VK, AS: conceptualization, study design, data validation, and writing – original draft preparation. AS, VK: collection of biological material AS: data curation and laboratory experiments. VK, AS: data analysis. VK: funding acquisition. VK, AS: revising and final approval of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

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CIRSIUM ARVENSE (ЕГІНДІК БОДЯК) – ФИТОПАТОГЕНДІ САҢЫРАУҚҰЛАҚТАРДЫҢ ТАБИҒИ РЕЗЕРВУАРЫ РЕТІНДЕ

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Abstract

Cirsium arvense – агроэкожүйелерде фитопатогенді саңырауқұлақтардың эпидемиологиясында маңызды рөл атқаратын, кең таралған және төзімді арамшөптердің бірі болып табылады. Бұл зерттеу Солтүстік Қазақстан облысындағы дәнді және бұршақ тұқымдас дақылдардың егістіктеріне жақын өскен C. arvense өсімдігімен байланысқан фитопатогенді саңырауқұлақтарды анықтауға бағытталды. Ауру белгілері бар өсімдіктердің (жапырақ, сабақ, гүлшоғыры) бөліктерінен саңырауқұлақтардың таза дақылдары бөлініп алынды. Қоректік ортада өсірілгеннен кейін, бөлінген изоляттарға рДНҚ-ның ITS аймағы негізінде молекулалық-генетикалық сәйкестендіру жүргізілді. Алынған нуклеотидтік тізбектер BLAST алгоритмі арқылы GenBank мәліметтер базасымен салыстырылды. Ең жиі кездесетін түрлер ретінде *Alternaria alternata* мен *Fusarium tricinctum* анықталды. MEGA 11 бағдарламалық жасақтамасының көмегімен Neighbor-Joining әдісі арқылы филогенетикалық ағаш құрастырылып, алынған изоляттардың эволюциялық туыстық деңгейі анықталды. Зерттеу нәтижелері C. arvense өсімдігі ауылшаруашылық дақылдары үшін қауіпті фитопатогендердің табиғи резервуары бола алатынын растады.

Кілт сөздер: *Cirsium arvense, фитопатогенді саңырауқұлақтар, ITS сәйкестендіру, Alternaria alternata, Fusarium tricinctum*

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БОДЯК ПОЛЕВОЙ (*CIRSIUM ARVENSE*) – КАК ПРИРОДНЫЙ РЕЗЕРВУАР ФИТОПАТОГЕННЫХ ГРИБОВ

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ABSTRACT

Cirsium arvense (бодяк полевой) является одним из наиболее распространённых и устойчивых сорняков, играющих важную роль в эпидемиологии фитопатогенных грибов в агроэкосистемах. Настоящее исследование было направлено на выявление фитопатогенов, ассоциированных с *C. arvense*, произрастающим вблизи посевов зерновых и зернобобовых культур в Северо-Казахстанской области. С визуально поражённых участков растений (листья, стебли, соцветия) были выделены чистые культуры грибов. После культивирования на питательных средах проводилась молекулярно-генетическая идентификация изолятов на основе анализа *ITS*-регионов рДНК. Полученные последовательности сравнивались с базой данных *GenBank* с использованием алгоритма *BLAST*. Установлено, что наиболее часто встречающимися видами являются *Alternaria alternata* и *Fusarium tricinctum*. С использованием программного обеспечения MEGA 11 построено филогенетическое дерево методом Neighbor-Joining, позволяющее определить эволюционные связи выделенных штаммов. Результаты подтверждают, что *C. arvense* может выступать природным резервуаром фитопатогенов, потенциально опасных для сельскохозяйственных культур.

Key words: *Cirsium arvense, фитопатогенные грибы, идентификация ITS, Alternaria alternata, Fusarium tricinctum*